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## SO-CALLED MIXED TUMORS OF THE SALIVARY GLANDS

WALTER H. SHELDON, M.D.

BOSTON

The nature and the site of origin of the so-called mixed tumors of the salivary glands are still obscure. The great variability in the character of the cellular components, among which cartilage and bone are found, as well as the peculiar appearance of the stroma, accounts for the uncertainty in regard to the nature of these tumors. Their origin from the salivary glands has been doubted, since neoplasms of similar appearance have been found in the skin, lacrimal glands and breast.

A complete review of the subject was given by Ahlbom in 1935.<sup>1</sup> He presented the various opinions which deal with the endothelial, mesenchymal or teratomatous character of these neoplasms. Such opinions are now of historical interest only. His conclusion favored the epithelial nature of these tumors. Ewing<sup>2</sup> agreed that these growths are epithelial. Concerning their origin he, as well as Ahlbom,<sup>1</sup> expressed the belief that some arise from the mucous or salivary glands while others appear to come from aberrant embryonal anlagen or possibly from remnants of the branchial cleft.

No essentially different opinions in regard to the nature and the origin of these tumors are recorded in the literature.

### NORMAL SALIVARY GLANDS

Although the histologic character of the salivary glands is well known, it might be permissible to outline briefly some of the principal features. This seems justified in view of the fact that one of the cellular components of the glands which normally is rather inconspicuous may assume a major role in some of the tumors of these organs.

By far the predominant element is the secreting epithelial cell, which is of cuboid shape. These cells are arranged in acini and produce either an albuminous or a mucous secretion. While some of the glands are

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From the departments of pathology of the Peter Bent Brigham Hospital and Harvard Medical School.

1. Ahlbom, H. E.: *Acta radiol.*, 1935, supp. 23, p. 1.

2. Ewing, J.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

composed exclusively of secreting cells of a single type, most of the glands are made up of mixed types and contain both albuminous and mucous secreting cells. All glands possess branching excretory ducts. The epithelium of those situated within the lobules appears to contribute to the secretion. The smallest intralobular ducts are called necks, isthmuses or intercalated ducts and are lined by low cuboid epithelium. The next larger and still intralobular ducts are referred to as striated tubules because of the peculiar striation of the columnar epithelium. The larger ducts present in the major salivary glands are lined by pseudostratified columnar epithelium which near the openings of the ducts changes into stratified squamous epithelium.

Around the acinous cells of all salivary glands, as well as around their isthmuses and striated tubules, other cells are present. These cells have been extensively studied by Zimmermann.<sup>3</sup> They are situated between the secreting epithelium and the basement membrane but inside the latter. Normally they are not numerous, and only their nuclei are easily visible. Their shape varies from stellate with several cytoplasmic processes to more elongated. Their nuclei are angular or more flattened and elongated. These cells possess fairly coarse longitudinal cytoplasmic fibrils which extend into the cell processes. They are arranged obliquely or almost circularly about the longitudinal axis of the acini or ducts and partly encircle the epithelial cells with their processes. The presence of syncytial connections between them is disputed. Zimmermann<sup>3</sup> denied the presence of such connections in human material.

These cells are called basket or basal cells. Their morphologic features and staining properties identify them as smooth muscle fibers, and as such they were first recognized by Engelmann.<sup>4</sup> This view is now universally accepted. The ectodermal origin of these cells appears to be undisputed. They are considered by the anatomists as contractile epithelial cells and are classified together with similar cells found in the mammary, lacrimal, ceruminous and sweat glands as myoepithelium. By their contractions they facilitate the propulsion of the excretion from the glands. In addition to the basket cells, spindle-shaped smooth muscle cells resembling more closely the myoepithelium of the sweat glands are found in similar locations around the isthmuses in some of the glands.

#### MATERIAL

Fifty-four examples of the so-called mixed tumor of the salivary glands were studied. The specimens had been fixed in Zenker's fluid. The following stains were used: eosin-methylene blue routinely, Mallory's phosphotungstic acid-hema-

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3. Zimmermann, K. W.: Die Speicheldrüsen der Mundhöhle und die Bauchspeicheldrüse, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 5, pt. 1, p. 130.

4. Engelmann, T. W., cited by Zimmermann.<sup>3</sup>

toxylin and Mallory's aniline blue in many instances and Weigert's elastic tissue stain occasionally. Hematoxylin-eosin was used sometimes to obtain better results on old material.

#### MICROSCOPIC OBSERVATIONS

On microscopic examination four groups could be distinguished among these tumors. These groups, however, were intimately related and overlapped each other.

*First Group.*—The tumors in the first group are irregularly lobulated, and each is surrounded by a dense connective tissue capsule. Some-

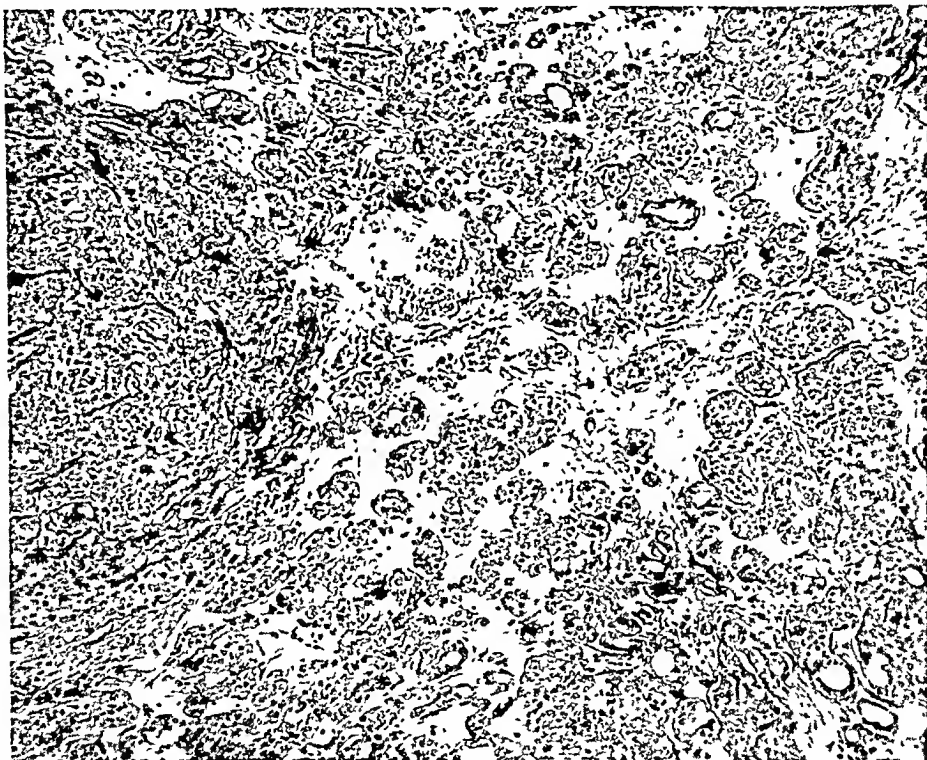


Fig. 1.—Adenoma of a parotid gland. Note the occasional small alveolar arrangement of the tumor cells and in some areas the loose and myxomatous appearance of the stroma. Eosin-methylene blue;  $\times 100$ .

times continuity with the adjoining uninvolved salivary gland is seen. They are markedly cellular, although in some areas the cells are more widely spaced. The tumor cells are of epithelial type and are generally arranged in solid clusters or anastomosing cords of varying width. Sometimes a small alveolar arrangement is present (fig. 1). In rare instances, larger cystic formations may be found. The tumor cells are mostly of polyhedral shape. Where an alveolar or cystic arrangement is present, they assume a cuboid or flattened shape. They are of medium size and possess round to oval vesicular nuclei. The fairly abundant



cytoplasm varies somewhat in appearance. In some tumors it is clear; in others it is finely granular and the granules are faintly eosinophilic (fig. 2 *A*). In still others, it is rather homogeneous and basophilic. A few of the alveolar structures contain small amounts of inspissated eosinophilic or basophilic material. There is no cellular pleomorphism, and mitoses are exceedingly rare.

Among the tumor cells a few other elements may be seen occasionally, but they are quite rare. These cells are of stellate shape with elongated or angular nuclei. Their cell body branches into several processes, and their eosinophilic cytoplasm exhibits longitudinal fibrils, which stain blue with the phosphotungstic acid-hematoxylin stain. These cells are situated at the periphery of the tumor cell clusters or cords but in close contact with them. Their processes partly encircle the tumor cells.

The connective tissue stroma varies in amount. In the densely packed cellular areas it is generally delicate and frequently rich in thin-walled blood vessels. Here it surrounds the clusters and cords of tumor cells after the fashion of a basement membrane. The rare stellate-shaped cells are always situated inside this membrane. In other areas the stroma is more abundant and frequently is dense, hyaline and acellular or is "moth eaten" and myxomatous in character. Some infiltration by lymphocytes and plasma cells, as well as some old and recent hemorrhage, may be seen occasionally. In some lobules, however, the stroma is present in such large amounts that the tumor cells proper are found only in rather small number and widely spaced.

In most of the neoplasms belonging to this group the already mentioned preponderance of the stroma is present throughout the entire tumor and represents a striking feature (fig. 2 *B*). The neoplastic cells are identical with those already described. They are present in widely spaced small or medium-sized collections or, frequently, they are scattered singly or in groups of three and four. A small alveolar arrangement is often seen, and the cells on the whole show marked secretory activity. The tumor cells are in general less altered and more abundant at the periphery of the individual lobules.

The stroma varies in character. It is poorly vascularized and stains mostly basophilic. In general, around the collections of tumor cells it is loose, fibrillar, markedly myxomatous and slightly to moderately basophilic. Where the tumor cells are few and scattered, the stroma is dense, hyaline, quite acellular and deeply basophilic. This appearance of the stroma coincides with changes in the tumor cells proper. Numerous secretion vacuoles appear in the neoplastic cells, causing marked swelling and ballooning of the cytoplasm and pyknosis of the nuclei. Thus the cytoplasm assumes a clear appearance, and the nucleus is flattened and displaced peripherally. In this manner, the isolated tumor

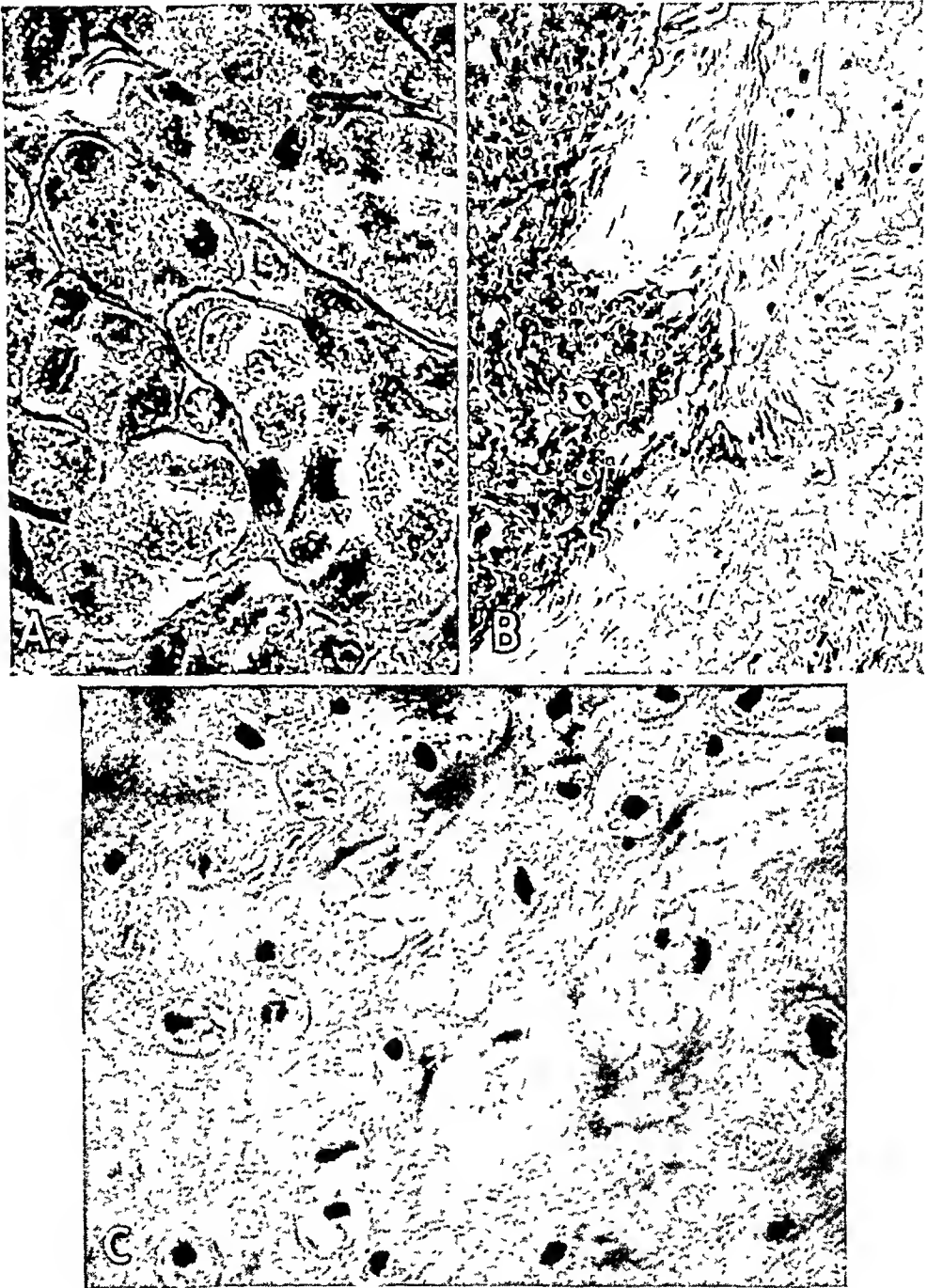


Fig. 2.—*A*, adenoma of a parotid gland. Note the conspicuous fine secretion granules in the cytoplasm and the mitosis, which are rare in this type of tumor. Eosin-methylene blue;  $\times 670$ . *B*, adenoma of a parotid gland. Note the abundant dense hyaline connective tissue stroma with some degenerating tumor cells scattered throughout it, giving the stroma its peculiar pseudocartilaginous appearance (compare with *C*). Eosin-methylene blue;  $\times 170$ . *C*, adenoma of a parotid gland. Note the varying stages of swelling and ballooning of the tumor cells as well as the density of the nuclei. Eosin-methylene blue;  $\times 1600$ .

cells are scattered through a background of markedly dense hyaline basophilic stroma, and the tissue assumes the appearance of cartilage (fig. 2 C). Sometimes masses of hyaline cartilage may be found in the same areas. Their true cartilaginous nature is recognized by the character of the dense homogeneous interstitial substance. This surrounds the cells proper, forming the so-called cartilage capsules. Most important, however, is the fact that in many instances its formation from the fibroblasts of the stroma can be seen at the periphery of these masses.

This group, therefore, is one of noncancerous tumors in which the neoplastic cells are of epithelial nature and tend to reproduce the normal epithelium of the salivary glands. Frequently the neoplastic cells undergo degeneration, which leads to a peculiar appearance of the connective tissue stroma. In many of these tumors the stroma is strikingly abundant and includes areas of metaplastic cartilage.

There is, however, a close relationship between the tumors of this and the tumors of the next group. This relationship is illustrated by the fact that a few of these tumors present in small areas a picture such as is seen in the neoplasms of the second group.

*Second Group.*—The tumors of the second group are composed of irregularly sized lobules, and each tumor is surrounded by a connective tissue capsule. An intimate relationship between the tumor tissue and the adjoining uninvolved salivary gland is seen in many instances. Some areas of the tumors are exceedingly cellular while elsewhere the neoplastic cells are quite sparse. There is considerable variation in the arrangement of the cells (fig. 3 A). Two different types of tumor cells are present, but both are generally in closest contact and relation with each other.

The cells representing the first type are definitely epithelial. They are the same cells which form the tumors of the first group. Their cytologic details are identical. No anaplastic cells are present, and mitoses are rare. Their arrangement is also basically the same as in the first group, but the formation of well defined small alveolar or duct-like structures is frequent and characteristic. In addition, the neoplastic cells may be found in small solid cords or clusters. Cystic dilatations of medium size occur in the alveolar or ductlike structures. Rarely, larger cystic formations with papillary infoldings are encountered. In the solid formations the tumor cells are polyhedral, while in the alveolar or duct-like structures they are cuboid. Where cystic dilation has occurred, they may be flattened out. Wherever they surround lumens they are present only in a single layer (fig. 3 B). Sometimes they may show extensive metaplasia into stratified squamous epithelium, occasionally with keratinization. Secretory activity in these cells varies from slight to marked. Granular or, more frequently, inspissated homogeneous

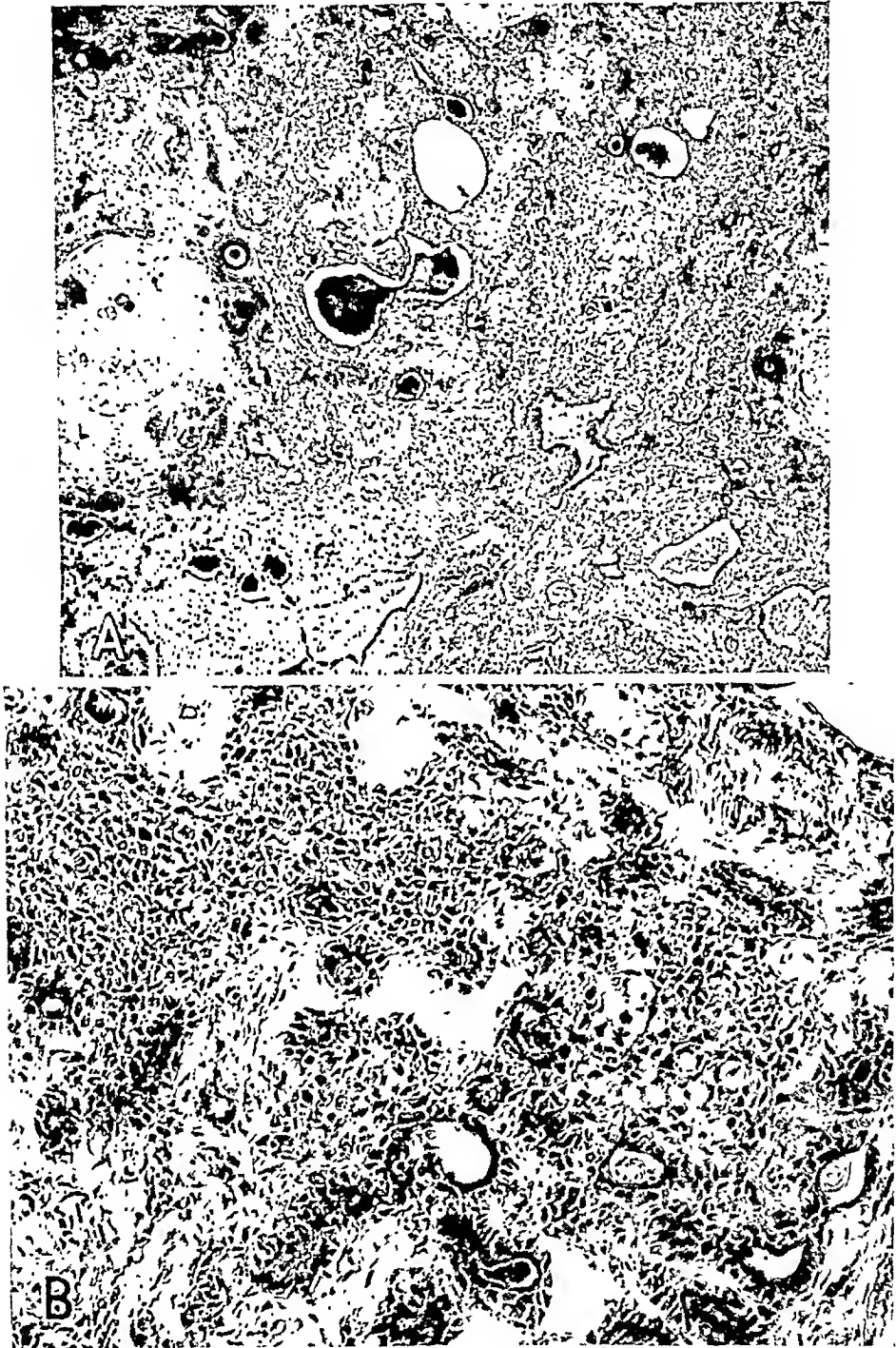


Fig. 3.—*A*, mixed tumor arising from the small buccal salivary glands of the cheek. Note the great variation in the arrangement, the numerous small and large ductlike structures with secretion, and the occasional squamous metaplasia and keratinization of the epithelium lining these structures. An area of true cartilage is seen at the left side of the picture. Eosin-methylene blue;  $\times 75$ . *B*, an area of the same tumor at higher magnification. The small ductlike structures lined by a single layer of epithelium and containing some secretion are surrounded by the prominent basket cells (see fig. 4 *B*). Basket cells are also seen in larger sheets. Eosin-methylene blue;  $\times 170$ .

eosinophilic or basophilic material may be found in the lumens. Where the secretory activity is most marked, the tumor cells may undergo degeneration identical with that described in the neoplasms of the first group.

The number of these cells varies considerably in the different tumors and even in the different portions of a single tumor. Sometimes they are quite numerous. Elsewhere they are few and widely spaced. On the whole they are considerably less numerous than the cells representing the second type, with which they are intimately connected in most instances.

The cells of the second type are the predominating tumor element (fig. 3 *B*). They display sometimes wide variation in shape. The stages leading to such variation in shape and appearance can be followed in almost every instance. These cells are of medium size. When widely separated from one another and situated in a loose background, they are of stellate shape because of the presence of several cytoplasmic processes. When they are closely packed or otherwise somewhat compressed, they appear as elongated fusiform elements. Similarly their nuclei, which are generally somewhat curved, vary from oval and elongated to rod shaped. The nuclei are quite dense and rich in chromatin. There may be occasional mitoses. These neoplastic cells possess a fair amount of eosinophilic cytoplasm, in which longitudinal cytoplasmic fibrils are seen (fig. 4 *A*). Their cytoplasm stains dark red with aniline blue and deep blue with phosphotungstic acid-hematoxylin in contrast to the epithelial components, which stain orange with aniline blue and brownish blue with phosphotungstic acid-hematoxylin. This difference in staining properties may be useful in those areas where the cells of the second type are of different appearance. In such areas, the cells are oval or round, and no longitudinal cytoplasmic fibrils can be made out. Their nuclei are oval and more vesicular. In a few rare instances large atypical nuclei are encountered. There are some mitoses.

These neoplastic cells also show variation in their arrangement. Where they are in contact with the epithelium of alveolar or ductlike formations they form the outer layer of these structures. This outer layer ranges from one to several cells in width. Here the cells show frequently a somewhat oblique palisade-like arrangement but are always situated inside the basement membrane (fig. 4 *B*). In other extensive areas these neoplastic cells are present in closely packed masses and may form interlacing bundles. Such an arrangement occurs where no alveolar or ductlike structures are present. Still elsewhere and also in large areas these tumor cells may be widely spaced and present the pattern of a tissue culture. Here the presence of anastomoses between the tumor cells is strongly suggested. Isolated islands of epithelial tumor com-

ponents may be encountered in all these areas. These epithelial cells are found isolated or in small groups of two or three. They are partly encircled by the cytoplasmic processes of a few intimately adjoining neoplastic cells of the second type.



Fig. 4.—*A*, the same tumor as in figure 3 *A*. A group of basket cells cut across almost longitudinally. The elongated fusiform shape of these cells is visible. Eosin-methylene blue;  $\times 670$ . *B*, the same tumor. Some of the ductlike structures are shown at high magnification. Note the inner single layer of epithelium and the prominent outer layer of basket cells arranged in palisade-like fashion. Eosin-methylene blue;  $\times 300$ .

The connective tissue stroma of this group of neoplasms displays the variations described in the first group. It ranges from delicate and fairly

vascular or loose, "moth eaten" and myxomatous to dense, hyaline and acellular. Where the stroma is of myxomatous appearance, it coincides with the previously described degenerative processes in the epithelial tumor components. Here, too, this degenerative process quite often produces a peculiar appearance of the stroma resembling that of cartilage. Areas of true cartilage and rarely of bone formed by metaplasia from the connective tissue are encountered (fig. 5 *A*).

Most of these tumors are surrounded by well defined connective tissue capsules. In a few instances, however, the capsule is incomplete and invaded (fig. 5 *B*). Occasionally extension of the tumor into the adjoining tissue may be seen. In these areas it is the epithelial component which appears to invade the adjoining tissue. It is accompanied by the elements of the second cell type, but these become less conspicuous. There is response of the stroma to the invading tumor elements. In 1 case invasion of nerve sheaths had taken place, and only in this case did the invading epithelial cells display an appreciable degree of anaplasia.

In conclusion, this group of neoplasms is composed of two different types of intimately connected cells. One type is represented by the same epithelial cells of the salivary glands which constitute the tumors of the first group. The other type is represented by cells which through their shape, appearance, staining properties and arrangement are identified as derived from the basket cells of the salivary glands. On the whole the latter cells are the predominating tumor element. Degenerative processes in the epithelial component may lead to a peculiar appearance of the connective tissue stroma. Metaplastic cartilage and bone derived from the connective tissue stroma may be found. Most of these tumors are noncancerous. Occasionally the basket cells display some anaplasia, but they do not show evidence of invasiveness. A few tumors show local invasion, and in 1 instance definite cancer was found. It is the epithelial component which becomes cancerous and invasive.

The same close relationship which connects the tumors of the foregoing two groups exists between those of the second and those of the third group. In areas the neoplasms of the second group display the cellular components and structural pattern characteristic of the tumors to be described next.

*Third Group.*—The tumors of the third group consist of small, generally solid nodules which are well encapsulated. The neoplastic cells are essentially identical with the basket cells of the tumors of the second group. On the whole they tend to be more frequently of elongated fusiform shape and are arranged in closely packed interlacing strands (fig. 6 *A*). In one of these tumors, however, the cells in large areas were of stellate shape, were rather widely spaced and displayed a structural pattern like that of a tissue culture (fig. 6 *B*). In these areas it



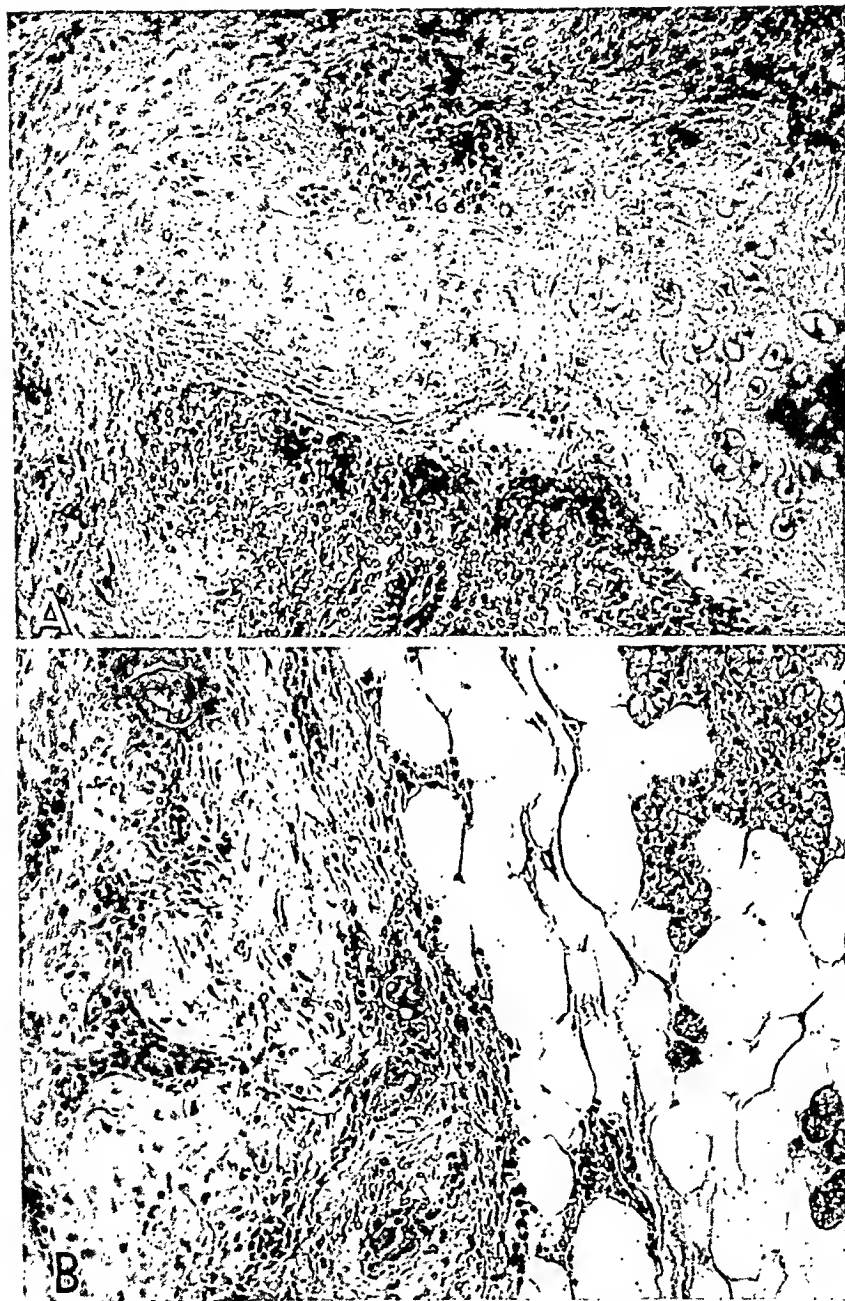


Fig. 5.—*A*, mixed tumor of a parotid gland. Note the area of metaplastic cartilage. The various stages in the formation of cartilage are well seen. Eosin-methylene blue;  $\times 170$ . *B*, mixed tumor of a parotid gland, locally invasive. The absence of a capsule between the tumor and the normal salivary gland tissue seen at the right is shown. At the upper left corner a ductlike structure surrounded by basket cells and characteristic of this tumor group is visible. Note that there is only a minimal stromal response where the tumor extends into the adipose tissue between the normal parotid lobules. Eosin-methylene blue;  $\times 170$ .



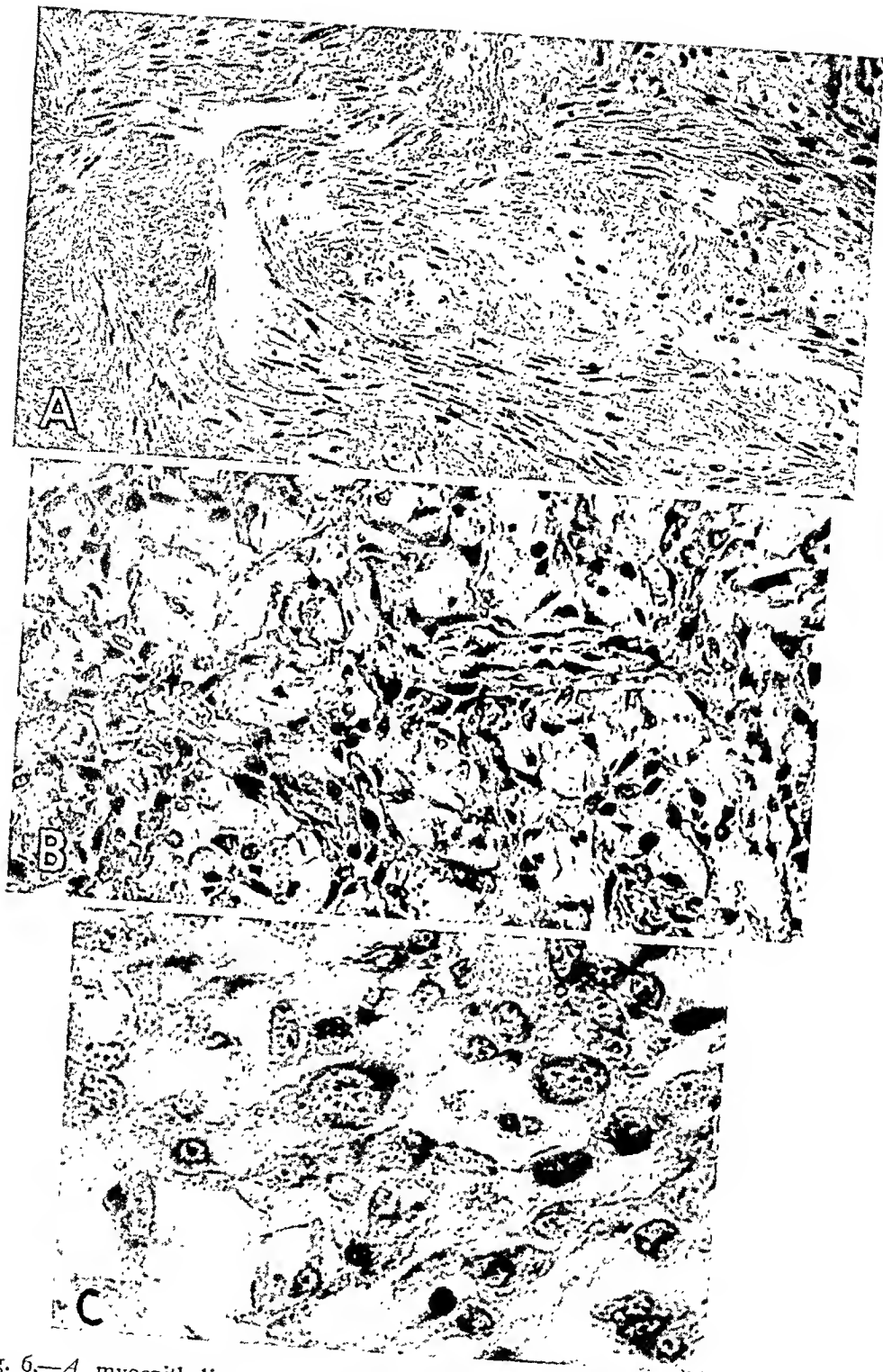


Fig. 6.—*A*, myoepithelioma of a parotid gland. The basket cells here resemble closely the usual smooth muscle fibers and are arranged in interlacing bundles. Some ductlike structures formed by epithelium are present in this tumor but not shown in this picture. Eosin-methylene blue;  $\times 170$ . *B*, myoepithelioma of a parotid gland. The basket cells form an anastomosing feltwork and show some cellular pleomorphism. Eosin-methylene blue;  $\times 310$ . *C*, the same tumor at higher magnification. The cellular pleomorphism of the basket cells is shown. Note the large atypical basket cell in the center. The patient was well and without recurrence three years after the excision of the tumor. Eosin-methylene blue;  $\times 670$ .

was evident that the cells anastomosed with one another by their cytoplasmic processes and formed a loose feltwork. In each tumor there are sometimes quite extensive areas in which the cells are large and round or oval, without longitudinal cytoplasmic fibrils. Some mitoses and cells with large atypical nuclei, as well as unusually large elements two or three times the size of the other cells, were found in one of these tumors (fig. 6 C).

Some epithelial cells are also present. They are arranged in small or medium-sized alveolar or ductlike structures. The cells and the structures formed by them are identical with those in the neoplasms of the first and second groups. In general they are few, and only in 1 instance were they present in appreciable number. Secretory activity is present in these cells but not to a significant degree. The alveolar or ductlike structures are surrounded by a basement membrane which separates them from the tumor cells proper. Only rare inconspicuous basket cells are present inside this basement membrane.

The connective tissue stroma is fairly well vascularized. It varies from delicate and small in amount to moderately dense, hyaline and abundant. It is nowhere of myxomatous character, and no metaplastic cartilage or bone is present. The connective tissue capsule varies in thickness from delicate to fairly wide. There is no invasion or penetration of the capsule.

In summary it may be said that these tumors are formed by cells of the second type, the basket cells of the salivary gland. Epithelial elements are also present but are few and do not participate in the formation of these tumors. Here the similarity between the basket cells and the smooth muscle cells of conventional type is most marked. Only in 1 instance did the basket cells display evidence of low grade cancer.

*Fourth Group.*—The tumors of the fourth group are related to those of the first group, of which they represent the cancer variant.

These neoplasms are composed of varying-sized lobules. They are incompletely or not at all encapsulated. They are markedly cellular, but in some areas the neoplastic cells may be rather widely spaced. The tumor cells are of the epithelial type (fig. 7 A). In many instances the cells are arranged in solid clusters or cords with some small alveolar structures visible here and there. An alveolar arrangement with occasional small or medium-sized cystic dilatations predominates in others. In 1 instance a number of large cystic spaces with papillary infoldings were found. Some of these papillary infoldings showed a secondary alveolar arrangement. The tumor cells where present in solid masses are polyhedral. Around the alveolar formations they are cuboid. Cellular pleomorphism varies from slight to marked. Metaplasia into stratified squamous epithelium with keratinization may be present but is rare. On the whole the cells possess fairly large, rather vesicular nuclei with

prominent nucleoli. Where the cells are markedly anaplastic two or three nucleoli may be present. Mitoses are frequent and often atypical. The cytoplasm is quite ample and shows the already mentioned variation from clear to finely granular eosinophilic or dense basophilic. There

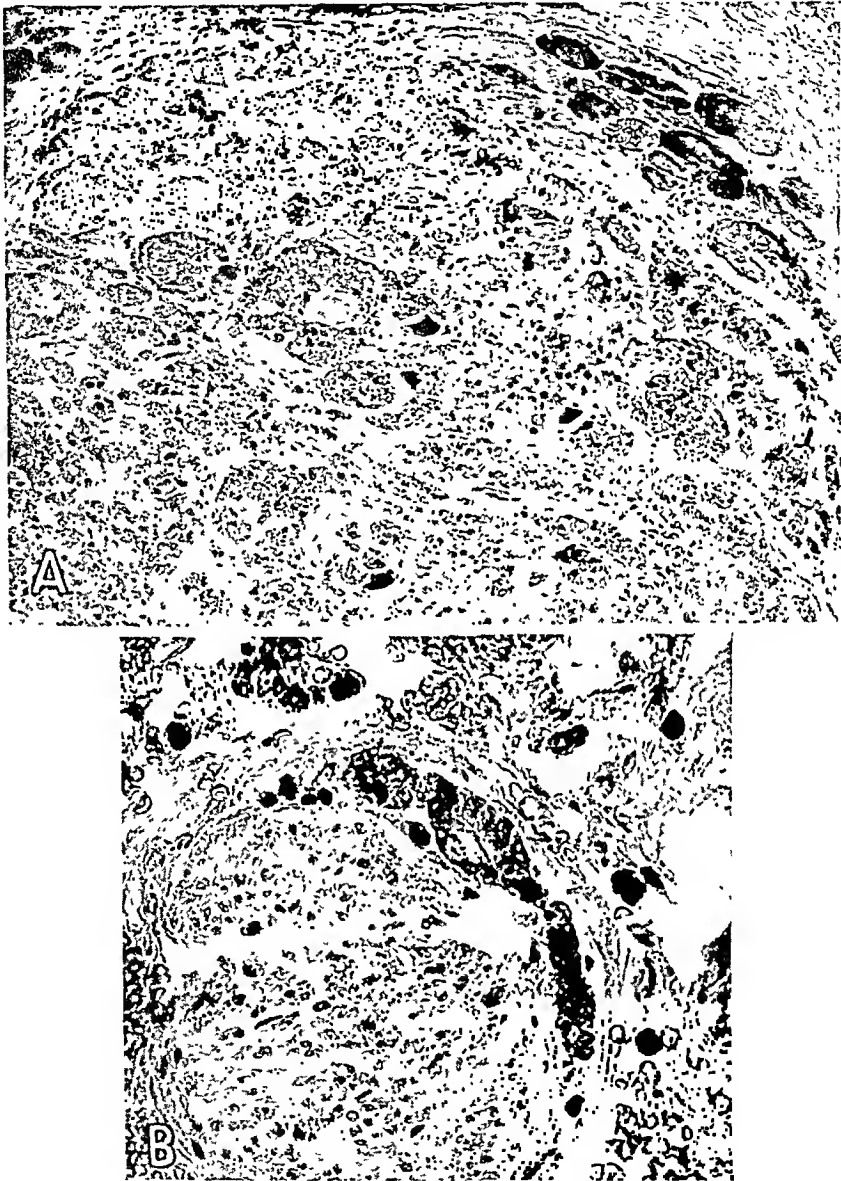


Fig. 7.—*A*, carcinoma of a submaxillary gland. The tumor is invading striated muscle seen at the right upper corner. Isolated muscle fibers in various stages of degeneration are incorporated in the tumor. The patient died of pulmonary metastases. Eosin-methylene blue;  $\times 100$ . *B*, carcinoma of a submaxillary gland. Invasion of the nerve sheath by tumor cells. Ten months later the patient was reported to have pulmonary metastases and to be moribund. Eosin-methylene blue;  $\times 300$ .

is evidence of secretory activity. In a few instances this leads to degeneration similar to that previously described, with ballooning of the tumor cells and signet ring appearance. Occasionally areas of necrosis are encountered. Extensive invasion of the adjoining tissue by the tumor has occurred frequently. Portions of uninvolved salivary gland tissue, muscle bundles and other components of the surrounding tissue may be found incorporated in the tumor at its periphery. Invasion of nerve sheaths is a common feature (fig. 7 *B*), and tumor cells are often seen in lymphatics and sometimes in small veins. Although most of these neoplasms show a high degree of anaplasia and invasiveness, such a high degree of canceration is not always present. A low grade of cancer with only local invasiveness and partial lack of encapsulation was seen in 1 instance.

Sometimes, although rarely, basket cells are encountered. They are present in about the same proportion as in the neoplasms of the first group and do not participate in the formation of the tumors.

In general the tumors have elicited a marked stromal response. This stroma consists of moderately well vascularized connective tissue which varies in amount and character. Sometimes it is rather meager, fibrillar and coarse, while elsewhere it is abundant, dense, hyaline and acellular. In a few areas coinciding with degeneration in the tumor cells proper the connective tissue stroma assumes a loose, myxomatous and slightly basophilic appearance, but this is not a prominent feature. No metaplastic cartilage or bone is found. The stroma may display some chronic inflammatory cell infiltration and occasionally evidence of old or recent hemorrhage.

In conclusion one may say that these are cancerous tumors of a purely epithelial nature which, like the neoplasms of the first group, arise from the epithelium of the salivary glands. The degree of canceration varies from local invasiveness to marked anaplasia. The latter is more common, and invasion of the nerve sheaths is frequent. The relationship to the tumors of the first group is enhanced by occasional degeneration of the tumor cells and subsequent changes in the connective tissue stroma. These changes, however, do not assume any major proportions.

#### STATISTICAL DATA

In tables 1, 2 and 3 the following data are shown: the distribution of the 54 tumors which form the basis of this report among the four groups; the frequency with which the various salivary glands are involved; the distribution of the tumors of each group in relation to the various glands and to the right and the left side, and the sex incidence.

From these tables it may be seen that the tumors of the second group are the most common, while those of the first group are next in

frequency, followed at some distance by those of the fourth group. The neoplasms of the third group are rare (table 1).

The parotid gland is the most commonly involved; the submaxillary gland less frequently. Involvement of the other sites, such as the inside

TABLE 1.—*Incidence of Noncancerous and Cancerous Neoplasms in the Four Groups and Distribution of the Tumors of the Four Groups with Reference to Sides and Sex*

	First Group	Second Group	Third Group	Fourth Group
Total number (54).....	19* (35%)	25 (46%)	3 (5%)	7 (13%)
Noncancerous.....	19 (100%)	20 (80%)	2 (67%)	
Cancerous.....	.....	5 (20%)	1 (33%)	7 (100%)
Side: Right.....	7† (39%)	7 (28%)	1† (50%)	4† (67%)
Left.....	11 (61%)	18 (72%)	1 (50%)	2 (33%)
Sex: Male.....	5 (26%)	6# (20%)	1¶ (50%)	4 (57%)
Female.....	14 (74%)	20§ (80%)	1   (50%)	3 (43%)

\* There was a marked predominance of the stroma in 12 cases (63 per cent).

† In 1 case the side was not specified.

# Two of these were malignant.

§ Three of these were malignant.

¶ In 1 case the sex was not indicated.

|| This tumor was malignant.

TABLE 2.—*Different Sites of the Tumors and Their Frequency of Involvement*

Total Number	Parotid Gland	Submaxillary Gland	Cheek	Epiglottis	Hard Palate
54	45 (83%)	6 (11%)	1 (2%)	1 (2%)	1 (2%)

TABLE 3.—*Incidence of Noncancerous and Cancerous Tumors of the Four Groups in the Different Sites*

Group	Parotid Gland	Submaxillary Gland	Cheek	Epiglottis	Hard Palate
First group.....	19 (35%)	.....	.....	.....	.....
Noncancerous.....	19 (100%)	.....	.....	.....	.....
Cancerous.....	.....	.....	.....	.....	.....
Second group.....	19 (35%)	3 (5%)	1 (2%)	1 (2%)	1 (2%)
Noncancerous.....	15 (79%)	3 (100%)	1 (100%)	1 (100%)	.....
Cancerous.....	4 (21%)	.....	.....	.....	1 (100%)
Third group.....	3 (5%)	.....	.....	.....	.....
Noncancerous.....	2 (67%)	.....	.....	.....	.....
Cancerous.....	1 (33%)	.....	.....	.....	.....
Fourth group.....	4 (7%)	3 (5%)	.....	.....	.....
Noncancerous.....	.....	.....	.....	.....	.....
Cancerous.....	4 (100%)	3 (100%)	.....	.....	.....

of the cheek, the epiglottis and the hard palate, is encountered only sporadically (table 2). An analysis of the distribution of the tumors of the four groups in relation to the various glands gives similar results (table 3). No tumor of the sublingual gland was present in this series.

The left side was more frequently the site except in the fourth group (table 1). Women were more frequently affected than men (table 1).

The age incidence ranged from the first to the eighth decade with a predominance in the fifth and the sixth decade.

## COMMENT

That all the 54 tumors of this series originated in the various salivary glands is definitely established.

The tumors of the first group are of a relatively simple nature. They are derived from the secreting epithelium of the salivary glands and are noncancerous. In a number of instances extensive degeneration of the tumor cells occurs and leads to a peculiar myxomatous and later pseudocartilaginous appearance of the connective tissue stroma. The basis of this degenerative process appears to be the normal secretory activity of the epithelium of the salivary glands, which, however, is reproduced by the tumor cells in a quantitative and probably also a qualitative abnormal manner. True cartilage formed by metaplasia from the connective tissue stroma may further complicate the picture.

These tumors are best classified as adenoma.

The neoplasms of the second group are more complicated, since two different types of cells participate in their formation. In addition to the secreting epithelium, the basket cells, which are a normal component of the salivary glands, contribute not only to the tumor but are actually the predominating cells. Although in a single tumor neoplastic cells of both types are found growing independently of each other, in large portions they display a quite characteristic relationship. This consists in small alveolar or ductlike structures in which the epithelium forms the inner or lining layer, while the basket cells, although in immediate apposition with the epithelium, constitute the outer layer. A basement membrane is present only peripherally to this outer layer. Thus these neoplasms are truly organoid. They reproduce in great detail the normal components and structure of the organ from which they arise, although in a distorted and exaggerated fashion. This distortion of the normal picture is accentuated by the pleomorphism of the basket cells as well as by the described degeneration of the epithelium with subsequent myxomatous and pseudocartilaginous appearance of the stroma. In addition, squamous metaplasia of the epithelium may occur. True cartilage or bone which arises by metaplasia from the connective tissue stroma further confuses the picture. Most of these tumors are noncancerous but a few are cancerous. In these the epithelium shows local invasiveness or even canceration, while the basket cells only suggest slight anaplasia.

The classification of these tumors is difficult. Since they are composed of neoplastic cells of two different types, consisting of the epithelial as well as of the basket cells, the term "mixed tumor" might be reserved for this group.

The tumors of the third group are relatively simple. They are formed by the basket cells alone. There may be some epithelial structures, but these are not an active part of the tumor proper. They rather represent remnants of the normal acini and ducts which have become

included in the tumor. In these neoplasms, no myxomatous or pseudocartilaginous appearance of the connective tissue stroma is seen, and no true cartilage and bone are encountered. This is to be expected since the peculiar changes in the stroma, and probably also the true cartilage and bone, are only the result of degeneration in the epithelial tumor elements. The basket cells display a considerable degree of pleomorphism. They may be fusiform and arranged in interlacing bundles, thus closely resembling the usual smooth muscle fibers. They may also be stellate shape with anastomoses forming a loose feltwork. Sometimes they are of round or oval shape and have lost their longitudinal cytoplasmic fibrils.

These neoplasms may be noncancerous or cancerous, but in this series they showed only a low grade of invasiveness. It is difficult to name these tumors, which do not fit any of the usual classifications. Since they are composed of the basket cells which belong to the contractile type of epithelial cells or myoepithelium, the term "myoepithelioma" may be indicated.

The tumors of the fourth group are also quite simple. They are invasive and arise from the secreting epithelium of the salivary glands in the same fashion as those of the first group. Degeneration of the tumor cells and resulting myxomatous or pseudocartilaginous appearance of the connective tissue stroma are sometimes present but not prominent, and no true cartilage or bone is encountered. The scarcity of these changes in the stroma may be attributed to the rapid growth of these neoplasms and to the lack of differentiation of their cells since most of the tumors in this group are highly anaplastic. Invasion of nerve sheaths appears to be their most common route of extension.

These tumors are best classified as carcinoma.

Although the four groups presented in this series show distinctive features, they are all related to each other and must be regarded as variations of a certain type of neoplasm which is frequently referred to as "mixed tumor of the salivary glands." It is probable that variations other than those described can occur. Borderline forms are present in all four groups and represent merely the connecting link between the different groups. They might be included in one or the other group according to the individual observer.

Much of the discussion about the nature of the tumors in question has arisen from the peculiar myxomatous and pseudocartilaginous appearance of the stroma which is so frequently encountered. The findings in this series indicate that this peculiar appearance of the stroma is only a secondary phenomenon and is caused by the secretion of the epithelial tumor cells with subsequent increase and degeneration of the connective tissue framework. This sequence of events had been observed by many investigators quoted by Ahlbom.<sup>1</sup> The presence of true car-

tilage and bone offered another difficulty in the understanding of these neoplasms. The explanation most commonly given, apparently endorsed by Ewing,<sup>2</sup> is that cartilage and bone are derived by direct transformation from the neoplastic epithelium. In contrast to this concept, the observations reported here indicate that true cartilage and bone are formed by metaplasia from the connective tissue stroma. Harvey, Dawson, and Innes<sup>5</sup> stated a similar belief.

The presence and the active participation of the basket cells in the formation of these tumors do not appear to have been recorded in the literature. In many of the so-called mixed tumors of the salivary glands the spindle or stellate shape of the neoplastic cells, their arrangement in anastomosing feltworks or interlacing strands and their characteristic relationship to the epithelial components have frequently been described and illustrated. These features have led to the description of a sarcomatous or an endotheliomatous appearance.

The resemblance to certain tumors of the skin has been recognized and acknowledged by such terms as "cylindroma," "basal cell carcinoma with hyaline stroma" or "adenoid cystic carcinoma." Because of this similarity it has been doubted that the salivary glands actually give rise to the neoplasms under discussion.

The resemblance of the tumors of the second and third groups in this series to some of the tumors of the sweat glands is striking. This resemblance is caused by the close embryologic relationship of the epithelia and by the presence of a second type of peculiar cells in both the salivary and the sweat glands. The peculiar cells are the basket cells in the salivary glands and the myoepithelium in the sweat glands. It has already been mentioned that the basket cells belong to the group of contractile epithelial cells. This group as a whole is classified as the myoepithelium. Sweat gland tumors formed by epithelial and myoepithelial components or by the latter alone have been described by Sheldon.<sup>6</sup> Neoplastic proliferation of the myoepithelium in breast tumors has been shown by Hamperl.<sup>7</sup> Myoepithelium is also known to be present in the lacrimal and ceruminous glands. Although no tumors of myoepithelial nature have been described in these locations the resemblance of neoplasms arising here to those of the salivary glands has been pointed out by Ahlbom<sup>1</sup> as well as by Tillé and Leroux-Robert.<sup>8</sup>

Thus the confusing variability of the so-called mixed tumors of the salivary glands may be accounted for by three factors. The first consists

5. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: *Debatable Tumors in Human and Animal Pathology*, London, Oliver & Boyd, 1940, chap. 3, p. 17.

6. Sheldon, W. H.: *Arch. Path.* **31**:326, 1941.

7. Hamperl, H.: *Virchows Arch. f. path. Anat.* **305**:171, 1939.

8. Tillé, H., and Leroux-Robert, J.: *Bull. Assoc. franç. p. l'étude du cancer* **27**:596, 1938.



in the neoplastic proliferation of cells belonging to the myoepithelium alone or of these cells together with the epithelium. The second factor is the myxomatous or pseudocartilaginous appearance of the stroma, which is secondary to the degeneration of the epithelial tumor cells. The third is the metaplastic formation of true cartilage and bone from the connective tissue stroma.

#### SUMMARY AND CONCLUSIONS

The so-called mixed tumors of the salivary glands include cancerous and noncancerous neoplasms composed of one or two different types of neoplastic cells.

Both types of cells are normally present in the salivary glands. One is represented by the secreting epithelium; the other, by the basket cells. The latter are peculiar smooth muscle cells which belong to the myoepithelium.

Some of these neoplasms arise from the epithelium. In these tumors an excessive and probably also qualitatively abnormal secretion produces a peculiar myxomatous and pseudocartilaginous appearance of the connective tissue stroma. True cartilage may be present and is formed by metaplasia from the stroma.

Other neoplasms arise from both the epithelium and the basket cells. These are truly organoid tumors which closely reproduce the normal components and structure of the salivary glands. Squamous metaplasia of the epithelium, myxomatous and pseudocartilaginous stroma and true cartilage and bone may be present.

A few neoplasms arise from the basket cells alone.

The presence of myoepithelial cells in these neoplasms accounts for the resemblance to tumors of the sweat glands, the mammary glands and probably also the lacrimal and ceruminous glands.

# GASTROINTESTINAL INVOLVEMENT IN LYMPHATIC LEUKEMIA

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NEW ORLEANS

The pathologic changes in the gastrointestinal tract in lymphatic leukemia have not been clearly set apart from those of other lymphoid diseases. There have been many descriptions under the name of pseudoleukemia gastrointestinalis. This, however, included many conditions of different natures.

It is our purpose to report here 2 cases of chronic lymphatic leukemia with marked gastrointestinal involvement, together with certain pathologic characteristics which might help to distinguish lymphatic leukemia from other lymphatic involvements of the gastrointestinal tract.

From Jan. 1, 1938 to Jan. 1, 1942, a total of 28 cases of leukemia which had been investigated by necropsy were available for study at Charity Hospital of Louisiana at New Orleans. Of these, 20 were instances of lymphatic and 8 instances of myelogenous leukemia. In the lymphatic group there were 13 cases of the chronic, 5 of the subacute and 2 of the acute variety, while in the myelogenous group there were 4 of the chronic, 3 of the subacute and 1 of the acute variety.

In the following report of the 2 cases only the essential data are recorded.

## REPORT OF CASES

CASE 1.—A white woman aged 53 was first admitted March 9, 1941, with splenomegaly and cervical adenopathy of two years' duration. Bloody diarrhea had been noted for the first time Dec. 9, 1939. Roentgenologic study showed marked hyperplasia of the mucosa of the stomach, sigmoid and rectum. The white blood cell counts ranged from 14,000 to 22,000; the percentages of lymphocytes from 59 to 70 and those of neutrophils from 30 to 41. A course of high voltage roentgen therapy was given, and the patient improved. An attack of bloody diarrhea appeared again Feb. 9, 1941, which was present at the time of admission.

The liver and the spleen were enlarged. No external lymph nodes were palpable. Two courses of high voltage roentgen therapy were given to the neck, the axilla and the groin.

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On May 13 the patient was much improved. Bilateral axillary and right epitrochlear nodes could now be felt.

The patient was readmitted November 18 with bloody diarrhea of six weeks' duration. Slight general adenopathy was present. Hematologic studies during her stay in the hospital gave results as follows:

	3/10/41	4/16/41	5/13/41	11/18/41
Hemoglobin, Gm. ....	4.7	9.8	8.8	5.8
Red blood cells per cubic millimeter ...	1,140,000	3,020,000	2,300,000	1,540,000
White blood cells per cubic millimeter...	72,000	14,800	8,250	17,850
Platelets per cubic millimeter.....	145,000	140,000	100,000	40,000
Hematocrit, per cent.....	18	31	26	18.8
Mean corpuscular volume, cubic microns	159	102	113	122
Mean corpuscular hemoglobin, micro-				
micrograms .....	41	32	38	37
Polymorphonuclears, per cent.....	14.5	9	11.5	5.0
Eosinophils, per cent.....		1	1.0	1.0
Monocytes, per cent.....		1	1.0	1.5
Lymphocytes, per cent.....	85.5	89	86.5	92.5

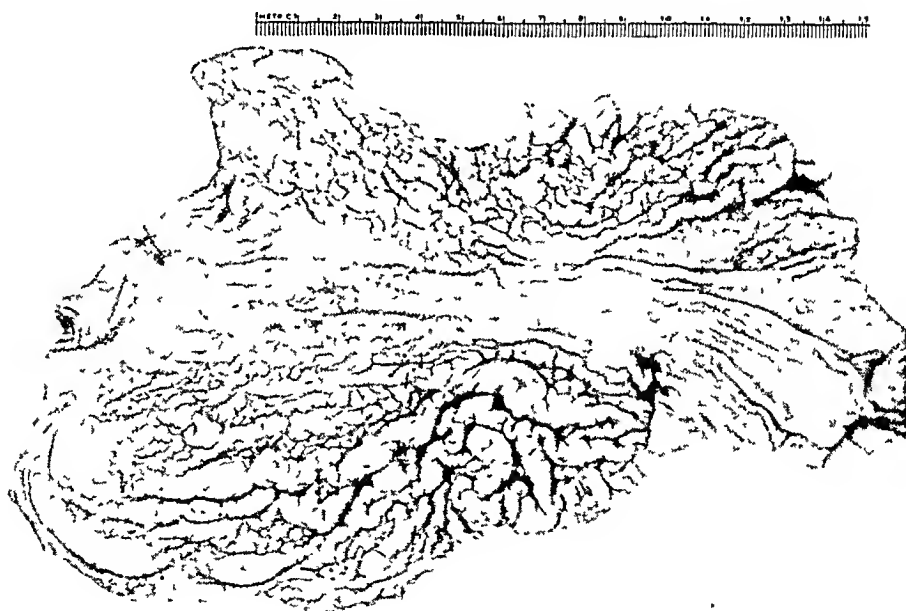


Fig. 1 (case 1).—Mucosa of the stomach thrown into irregular huge convoluted folds. Small superficial ulcers can be seen.

During her last stay in the hospital the peripheral blood showed severe anemia of the hyperchromic macrocytic type. The patient died December 2.

Autopsy revealed only a few discrete palpable nodes in the cervical region. However, the para-aortic, pancreatic, mediastinal and mesenteric nodes were enlarged but discrete.

The esophagus showed no gross change. The stomach showed involvement in its entirety. The mucosa was thrown into huge irregular folds, which resembled the convolutions of the brain. They were present throughout the entire stomach

but were most marked on the greater curvature and toward the pylorus, where they terminated abruptly. Cut sections showed no increase in thickness of the muscular wall, and gross inspection showed only involvement of the mucosa of the stomach. Here and there were many superficial ulcers up to 0.5 cm. in diameter with hemorrhagic areas (fig. 1).

The duodenum and the small intestine showed no change. The mucosa of the large intestine exhibited a change similar to that of the gastric mucosa and was thrown into thickened irregular folds. These also contained numerous ulcers, measuring 0.2 to 0.8 cm. in diameter. The muscular and serous coats were intact (fig. 2).

The liver and the spleen weighed 1,740 and 410 Gm. respectively, and gross examination revealed the typical appearance of leukemic infiltration. The bone marrow was viscid and grayish red. The remainder of the organs revealed no distinctive pathologic change. Post mortem the bone marrow showed replacement

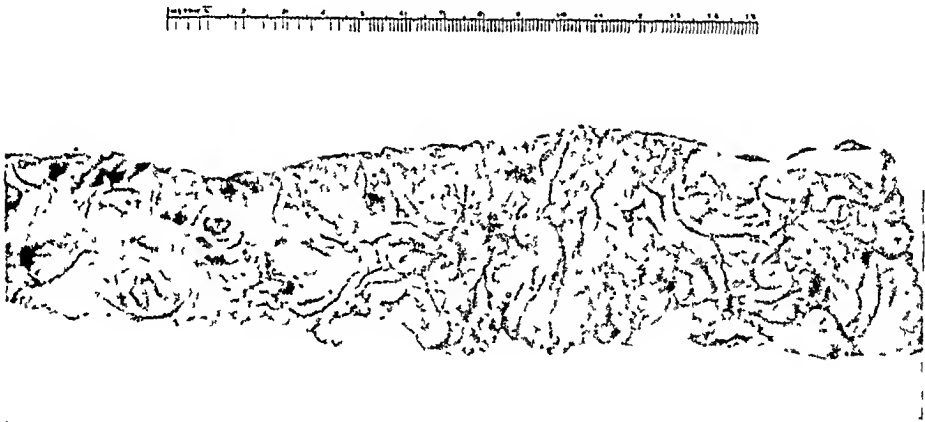


Fig. 2 (case 1).—Portion of the rectum and sigmoid colon showing involvement of the mucosa. Many ulcers are present.

with lymphocytes. The histologic picture of the lymph node, the spleen and the liver was that of chronic lymphatic leukemia.

CASE 2.—A Negro woman aged 39 was admitted July 28, 1938 with splenomegaly of four years' duration. The hemoglobin was 70 per cent, and the total white cell count was 294,000. In 1934 and again in 1937 high voltage roentgen radiation was given over the splenic region. During 1938 she received a total of 1,015 roentgens (r) over the spleen.

She was readmitted July 10, 1939, complaining of weakness, nausea and vomiting of twelve months' duration. The total white cell count ranged from 39,850 to 57,600.

She was finally admitted October 25, complaining of severe pains in both upper quadrants of the abdomen and of bleeding from the gums and rectum of four weeks' duration. The stools were tarry during her stay in the hospital.

Examination showed no general lymphadenopathy but showed enlargement of the spleen. There was bleeding from the mouth, nose, anus and vagina. A total

of 800 r was given over the spleen. Hematologic studies during her stay in the hospital gave the following results.

	10/26/39	11/21/39
Hemoglobin, Gm. ....	4.0	2.3
Red blood cells per cubic millimeter.....	1,800,000	670,000
White cells per cubic millimeter.....	96,000	90,000
Platelets per cubic millimeter.....	.....	50,000
Hematocrit, per cent.....	13	7
Mean corpuscular volume, cubic microns.....	72	104
Mean corpuscular hemoglobin, micromicrograms.....	22	34
Polymorphonuclears, per cent.....	12	2
Immature lymphocytes, per cent.....	72	98
Mature lymphocytes, per cent.....	16	.....

A diagnosis of chronic lymphatic leukemia with acute exacerbation was made. She died Nov. 11, 1939.

Autopsy revealed small lymph nodes in the cervical and inguinal regions. No other nodes were palpable.

The esophagus showed no gross change. The entire mucosa of the stomach was thrown into large irregular folds, and many of the rugae measured 3 mm. in thickness. This process terminated at the pylorus. Cut sections revealed no thickness of the muscular wall but only involvement of the mucosa. The remainder of the gastrointestinal tract showed no change.

The liver and the spleen weighed 2,125 and 450 Gm. respectively, and their appearance was typical of lymphatic leukemia. The remaining organs showed no pathologic change.

The histologic study of these cases revealed the usual picture of lymphatic leukemia. In the following histologic description emphasis is placed on the gastrointestinal tract.

In both cases the involvement of the stomach consisted of an infiltration of the mucosa and submucosa by round uniform cells, densely packed. The irregular edematous swellings of the submucosa together with the marked cellular infiltrations of the mucosa produced excessive exaggerations of the usual mucosal folds (fig. 3). In the more affected areas the mucosal glands showed atrophy and in some places actual ulceration.

The infiltration consisted of round cells which were of medium size (8-10 microns) and had a small amount of cytoplasm and a relatively large nucleus with coarse chromatin particles and often irregular nucleoli. Among them there were a few paler cells of oval shape (12-15 microns), and their nuclei were poor in chromatin. Between these cells there was often a fibrillar network. The blood vessels of the submucosa were numerous, distended and filled with red cells.

In the first case the large intestine was involved in its entire length and showed a similar histologic appearance with the exception of more frequent ulceration. In the second case the stomach alone was involved.

The marrow sections and imprint preparations, stained with hematoxylin and eosin as well as with Wright-Giemsa stain, showed complete replacement of marrow by lymphocytes (fig. 4). The spleen, the liver and lymph nodes showed the typical histologic picture of chronic lymphatic leukemia.



Fig. 3 (case 1).—Stomach wall revealing the characteristic lymphocytic infiltration of the mucosa and submucosa and the sharp demarcation of this process from the muscularis. The submucosa shows edema and vascular dilatation.  $\times 10$ .

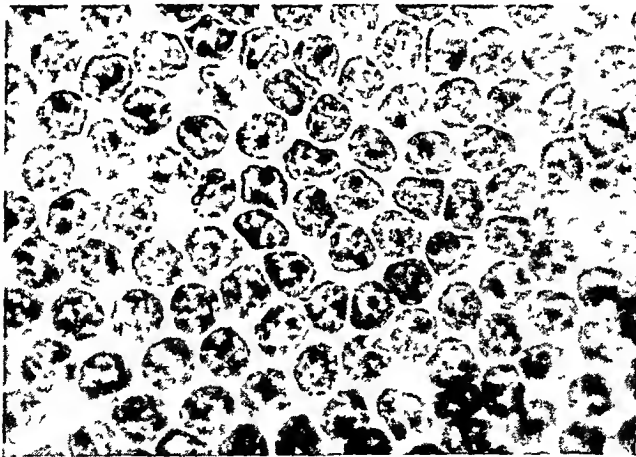


Fig. 4 (case 1).—Imprint preparation of sternal marrow stained by Wright-Giemsa stain, showing complete replacement by lymphocytic cells.  $\times 800$ .

## LITERATURE

The earliest and most classic descriptive effort was made by Briquet in 1838 and published in Cruveilhier's *Atlas of Anatomy*.<sup>1</sup> He described a case in which the mucosa of the stomach and large bowel was thrown into folds like "cerebral convolutions." We know of no better description or classic designation to depict this lesion. This was followed by many presentations of obscure heterogeneous conditions that were referred to as pseudoleukemia gastrointestinalis.

One of the first attempts to clarify this heterogeneous group was that by Wells and Maver in 1904.<sup>2</sup> They collected 7 cases, to which 1 of their own was added, which conformed to Briquet's descriptions. Ikeda<sup>3</sup> in 1931 thoroughly reviewed the literature and studied the incidence of gastrointestinal involvement in 78 cases of leukemia that came to autopsy. Among these 51 were instances of lymphatic and 26 instances of myelogenous leukemia. Gastrointestinal lesions as definite infiltrations occurred in 2 cases of the lymphatic variety, and in 1 case of myelogenous leukemia the gastrointestinal tract showed "local thickening." In 1931 Boikan<sup>4</sup> studied the gastrointestinal changes in 14 cases of leukemia that were investigated post mortem; 11 of these were cases of myelogenous and 3 were cases of lymphatic leukemia. In 1 case of the chronic lymphatic variety the typical brain-like convolutions were noted. In this case the mucosa and submucosa alone were infiltrated with lymphocytes.

Because of the confusion in the literature concerning this subject, it was thought advisable to assemble only those cases of chronic lymphatic leukemia which were proved to have been such by the data obtained at necropsy. Since Boikan and also Ikeda reviewed the literature up to 1931, only the subsequent cases were collected. In all except Kramer's case<sup>5</sup> the stomach was involved and showed a gross appearance similar to that in our cases. His patient for some time had aleukemic leukemia and died from ileus. Three areas of intussusception were present, due to large Peyer's patches that nearly occluded the lumen. Before death the peripheral blood picture revealed leukemic changes.

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1. Briquet, M.: *Maladies de l'estomac et des intestins*, in Cruveilhier, J.: *Anatomie pathologique du corps humain*, Paris, J. B. Baillière, 1838, vol. 2, no. 34, pp. 1-6.

2. Wells, H. G., and Maver, M. B.: *Am. J. M. Sc.* **128**:837, 1904.

3. Ikeda, K.: *Am. J. Clin. Path.* **1**:167, 1931.

4. Boikan, S. W.: *Arch. Int. Med.* **47**:42, 1931.

5. Kramer, K. W.: *Centralbl. f. allg. Path. u. path. Anat.* **60**:272, 1934.

Jörgenson<sup>6</sup> reported the case of a man aged 56 who was admitted with cervical adenopathy of one month's duration. The total white cell count was 74,100, with 81 per cent lymphocytes. The observations at necropsy were similar to those in our cases, with marked involvement of the mucosa of the stomach and large intestine. The muscularis was not involved in the process. Edema of the submucosa was present with marked vascular dilatation.

De Jongh's<sup>7</sup> case was that of a man 66 years of age who complained of bloody diarrhea for two years. The white blood cell count ranged from 18,000 to 23,000, with 57 to 60 per cent lymphocytes. A clinical diagnosis of "pseudoleukemia gastrointestinalis" was made. Necropsy revealed changes in the stomach and the large intestine identical with those in our first case. Histologically, only the mucosa and the submucosa were involved.

In Lüdin's<sup>8</sup> case biopsy of a lymph node was considered to have demonstrated lymphatic leukemia. Before death the white cell count was 38,800, with 78.3 per cent lymphocytes. At postmortem examination the gastric mucous membranes were thrown into huge folds up to 1 cm. in thickness. Microscopically, only the mucosa and the submucosa were involved.

Steinbrinck's<sup>9</sup> second case was that of a woman 70 years of age who complained of epigastric pain. At autopsy a diagnosis of aleukemic lymphatic leukemia was made. Infiltrations of the stomach were present.

The case of Touw and Graafland<sup>10</sup> was that of a white woman 74 years of age with severe hyperchromic macrocytic anemia. Tumor nodules were present in the scalp. Examination at necropsy revealed the mucous membranes of the stomach thrown into huge folds. Destruction of bone was seen in the cranium, ribs, sternum and tibia. They believed that this was a case of aleukemic lymphatic leukemia.

#### COMMENT

Most of the aforementioned authors emphasized the involvement as occurring only in the mucosa and submucosa. This was a distinctive feature in both our cases. In Ikeda's and Boikan's as well as in our own series of cases of leukemia, this change was present only in the

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6. Jörgenson, V. J.: *Ugesk. f. læger* **97**:327, 1935.

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8. Lüdin, M.: *Röntgenpraxis* **5**:816, 1933.

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lymphatic and not in the myelogenous variety. The diffuse involvement of the mucosa and submucosa, without further aggression, is a distinguishing feature in the majority of cases of lymphatic leukemia. However, similar gross changes may occasionally occur in the less aggressive types of lymphosarcoma.

#### SUMMARY

Two cases of chronic lymphatic leukemia with marked gastrointestinal changes were observed among 20 cases of lymphatic leukemia which came to necropsy. The characteristic gross and microscopic appearances have been described.

## ELASTIC TISSUE

### III. RELATIONS BETWEEN THE STRUCTURE OF THE AGING AORTA AND THE PROPERTIES OF THE ISOLATED AORTIC ELASTIC TISSUE

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One approach to the study of elastic tissue in the human aorta is based on simultaneous analyses of the intact vascular wall and the elastic system isolated from the vascular wall. The first step in this approach was the development of a method for the isolation of elastic networks without undue loss of their native properties.<sup>1</sup> The second step was a comparison of the measurements of extensibility, retractility and tensile strength of the purified elastic networks with corresponding measurements of the wall of the aorta from which the networks were isolated.<sup>2</sup> The mechanical and dynamic behavior of the isolated systems did not correspond with the behavior of the intact vascular walls. It seemed reasonable to search for causes of the divergent behaviors by recourse to studies of microscopic structure. The present report is concerned with the morphologic aspect of the problem as presented by 34 aortas ranging in age from 0 to 77 years.

#### DETERMINATIONS OF THE QUANTITY OF ELASTIC TISSUE

The quantity of elastic tissue in each of the 21 aortas was determined by a method which has been described.<sup>1</sup> Values were obtained for the loss of weight of each aortic wall at the end of twenty-four, forty-eight and seventy-two hours of extraction. In each instance a constant loss of weight was reached at the end of seventy-two hours. The residues, composed principally of intact elastic systems, possessed an approximate standard degree of purity.

The average quantity of elastic tissue in the series of 21 aortas was 37.9 per cent, with maximum deviations of  $+5$  and  $-9$  per cent (table 3).

Thirteen aortas were not analyzed for content of elastic tissue. In estimations of tensile strength the stated average value of 37.9 per cent was used as an approximation to the probable quantity.

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From the Department of Pathology of Cornell College of Medicine.

1. Hass, G. M.: Arch. Path. 34:820, 1942.

2. Hass, G. M.: Arch. Path. 34:971, 1942.

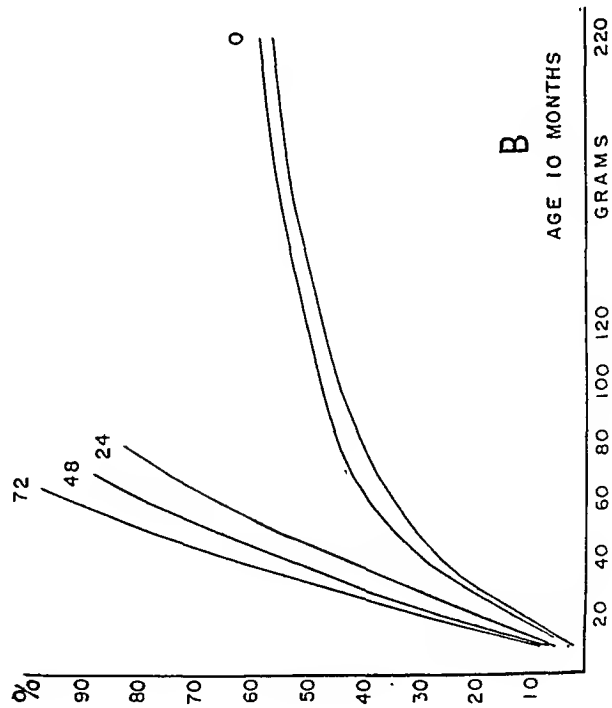
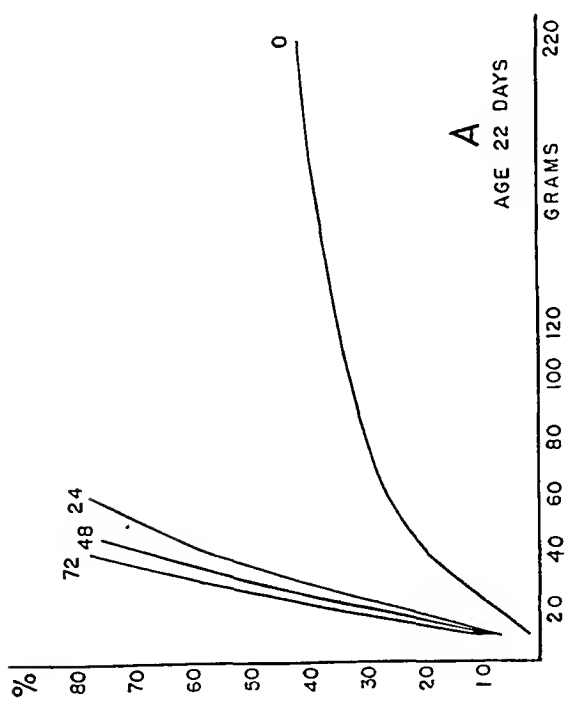


Figure 1

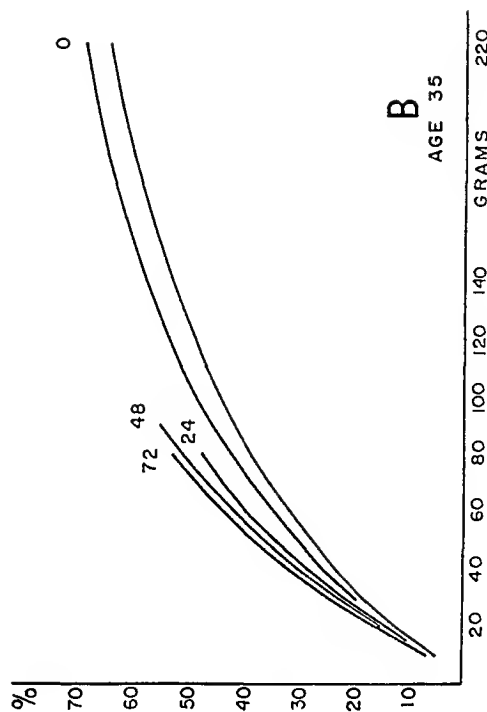
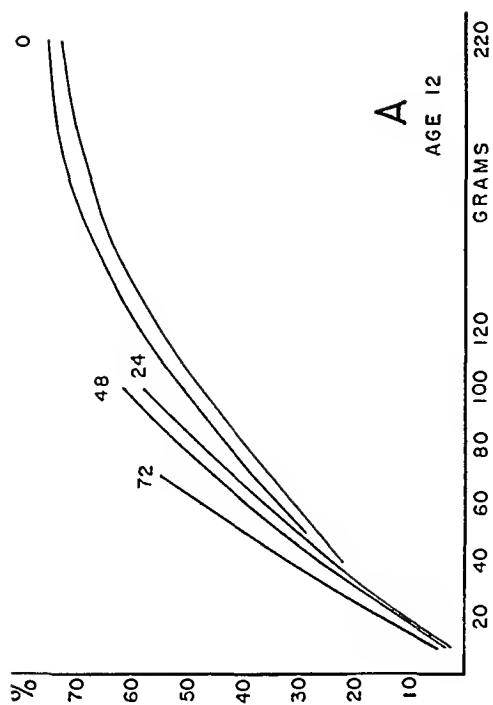


Figure 2

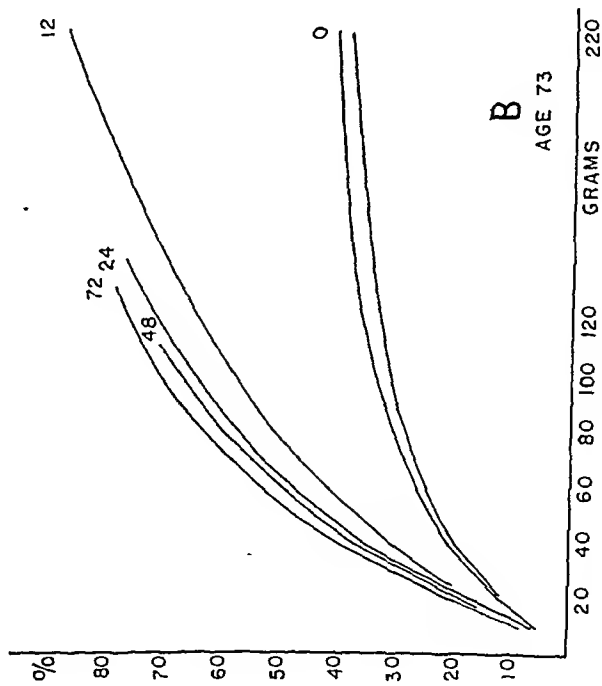
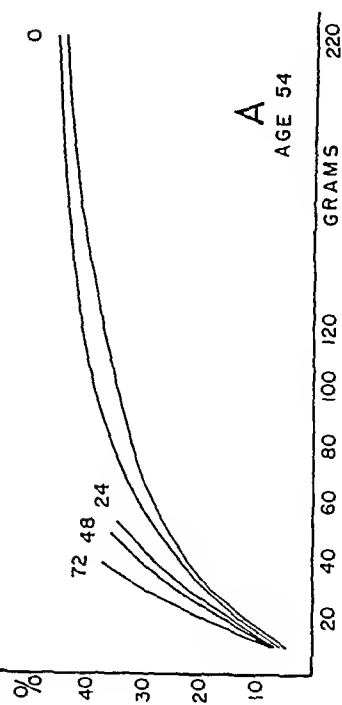


Figure 3

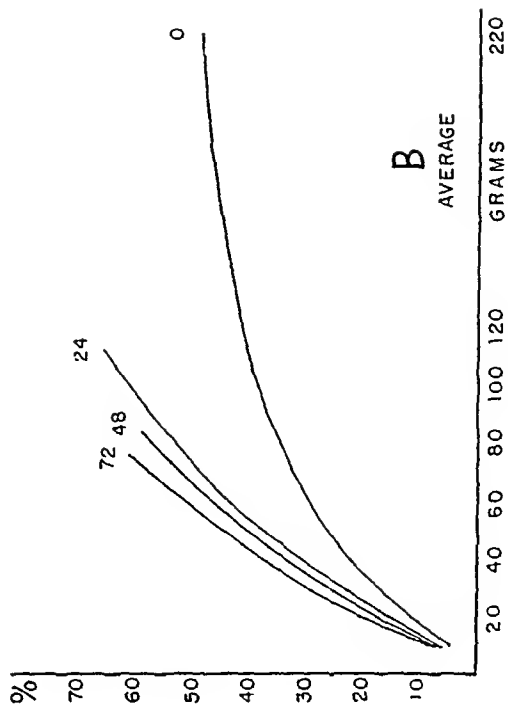
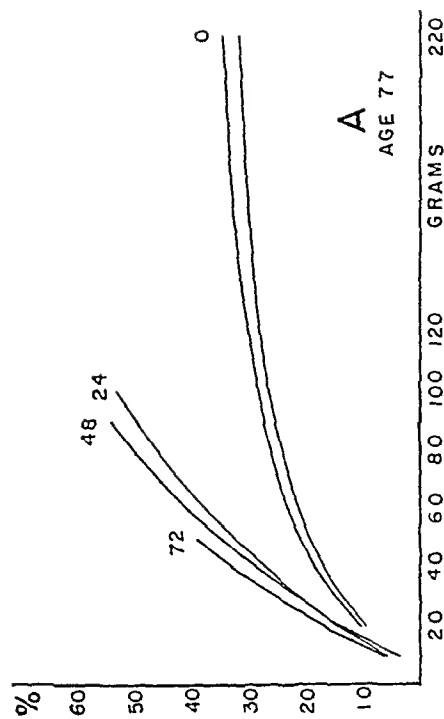


Figure 4

EXPLANATION OF FIGURES 1 TO 4

In each graph the extension of the vascular rings is plotted on the ordinate in percentage of the resting circumference. The load is plotted in grams along the abscissa. The number at the end of each curve pertains to the time of purification of the elastic networks in hours. When two curves at zero hours' extraction are present, they represent the minimum and maximum extensibility curves of the series of intact vascular rings used for extraction. The zero curves do not terminate, because the maximum load of 220 Gm. was never sufficient to rupture the vascular rings. All other curves terminate at coordinate points of near maximum extension and breaking load.

## MEASUREMENTS OF EXTENSIBILITY

The methods by which measurements of extensibility were made have been described.<sup>2</sup>

The initial measurements of the fresh unextracted aorta were made at 5 or 10 Gm. increments of load up to a weight of 220 Gm. The elongation under a load of 220 Gm. was arbitrarily taken as a standard measure of near maximum extension.

The curves of extension of a few intact vascular rings are shown in figures 1 to 4. These figures show that the shapes and amplitudes of the curves of extensibility of a series of contiguous rings from the same aorta are similar. Also, there is a close resemblance among the forms of the curves of extension of different aortic walls. The amplitudes of the curves, however, of the several aortas are different.

Though these curves have not been analyzed by mathematical methods, direct inspection discloses an approximate logarithmic form.

After the extensibility of intact vascular rings had been measured, all components except elastic tissue were extracted.<sup>1</sup> Satisfactory purification of the elastic tissue required extraction for seventy-two hours, but vascular rings were routinely studied after twenty-four, forty-eight and seventy-two hours of extraction.

At the three successive stages of purification, the extensibility of the residues of vascular rings was measured at increments of 5 or 10 Gm. of load up to a variable breaking load. The details of the method have been described.<sup>2</sup>

The data in the graphs and tables show that the extensibility of isolated elastic networks cannot be predicted from knowledge of the extensibility of the intact vascular walls from which they are obtained. The curves of all the intact walls are approximately logarithmic. The curves of the isolated elastic systems of young aortas are nearly linear (figs. 1 and 2 *A*). The curves of aged systems resemble arcs of circles or segments of sine curves (figs. 2 *B*, 3 and 4 *A*).

Each graph shows that prolongation of the extraction of similar aortic rings is accompanied by an increase in the slope of the extensibility curve. This is due partly to the extraction of nonelastic components which possess a measurable degree of tensile strength. The restraining action of these components is negligible after forty-eight hours' extraction.

A second factor in the increasing slope of extensibility curves during extraction is the degradation and solution of elastic tissue. The probable rate of solution is 2 to 5 per cent every twenty-four hours. The rate of degradation from a physical point of view is somewhat greater since the decrease in tensile strength in the forty-eight to seventy-two hour interval is about 10 per cent.

Despite the decrease in tensile strength, maximum extensibility of networks remains fairly constant during the twenty-four to seventy-two

hour interval. This is represented by the end points of the twenty-four, forty-eight and seventy-two hour curves. These points are important representations of the quality of the elastic tissue. The position of these points when plotted vary greatly among the thirty-four isolated systems. One variable which influences the positions of the points is the cross-sectional area of each elastic ring. If cross-sectional areas are adjusted to a standard value, there is a close correspondence of the slopes of all curves. Hence, it may be concluded that if an intact strand of elastic tissue from an infantile aorta extends 1 cm. under a tension of 1 Gm., an intact strand of equal dimensions from an aged aorta will likewise extend 1 cm. under a tension of 1 Gm. But, though the slopes of all curves when corrected are nearly equal, their lengths are unequal. The inequalities are functions of fragility, as represented by tensile strength.

#### MEASUREMENTS OF RETRACTILITY

Four sets of measurements were necessary for calculation of retraction: first, the circumference of the intact aortic ring before application of load; second, the circumference of the ring after removal of load; third, the circumference of networks at successive stages of purification; and fourth, the circumference of purified networks after application and removal of load.

All data are not recorded in the tables. In general, all rings from the same vessel had similar retractility though variations were encountered among the several vessels. The capacity of all vascular rings to retract increased during extraction up to the stage of suitable purification of networks. The values obtained after zero and seventy-two hours of extraction are recorded in table 1.

Two types of retractility were noted. The first type occurred spontaneously in the absence of applied load during neutralization of the acidic isolated networks. The second type occurred after application and removal of loads.

The spontaneous retraction on neutralization of networks is of two varieties. The first type is related to extension of elastic networks under the influence of a chemical force. The second type is related to removal of nonelastic components from the vessel wall.

When aortic rings at zero load are placed in 89 per cent formic acid, there is gradual elongation. This elongation is of the same order of magnitude as the extension realizable by application of mechanical tension. The chemical extension is maintained during purification of the elastic tissue. On neutralization of the purified networks, there is spontaneous retraction. In the case of young aortas the neutralized elastic tissue returns to the original length. In several other instances the neutralized rings retract to a circumference which is less than that

of the intact aortic wall from which they were obtained. The data in table 1 show that this excess spontaneous retraction is principally a characteristic of aged networks, especially those obtained from dilated and tortuous aortas. I believe that this is not an intrinsic property of aged elastic tissue but is a result of removal of constraints which maintain elastic networks of the aged vessel under constant tension after

TABLE 1.—*Retractility of Each Intact Aorta and Each System of Elastic Tissue\**

Number	Age, Yr.	Initial Circumference of Intact Aorta	Circumference of Isolated Elastic Rings	Permanent Elongation of Intact Aorta, per Cent	Permanent Elongation of Elastic Rings, per Cent
50	0	1.1	1.1	10.9	3.6
24	.03	1.6	1.6	4.4	3.8
44	.06	1.6	1.6	15.6	1.9
54	.06	1.5	1.5	10.7	2.7
35	.80	1.8	1.7	5.6	2.4
23	2.5	2.8	2.8	5.7	3.6
38	12	3.5	3.5	11.4	1.7
28	23	3.4	3.3	8.8	3.0
56	24	3.8	3.8	8.4	1.6
55	28	3.8	3.7	11.6	3.8
42	35	5.0	4.7	8.0	3.4
41	41	5.1	4.9	7.1	2.4
52	42	4.5	4.5	3.1	3.1
34	44	4.6	4.8	7.6	4.2
39	45	5.4	4.9	9.8	4.9
53	50	4.8	4.6	6.7	1.7
47	51	5.2	4.9	8.5	4.1
36	51	5.3	5.1	7.6	4.3
45	53	5.4	5.3	8.9	4.5
26	53	4.7	4.5	16.2	4.4
43	54	6.0	5.6	10.0	1.4
27	54	4.9	4.7	10.2	4.7
37	54	6.1	6.1	7.9	3.6
51	58	5.3	5.1	7.6	4.3
40	58	5.4	5.4	11.1	1.5
32	62	4.8	4.5	8.3	1.0
48	62	6.2	5.7	10.3	3.4
25	63	4.7	4.4	9.6	1.0
33	67	4.5	4.5	6.7	3.6
31	70	5.7	5.2	7.9	3.9
46	71	7.2	6.4	8.3	6.3
30	73	4.9	4.7	8.2	5.1
29	74	4.5	4.1	8.9	4.9
49	77	6.8	6.1	11.8	3.2
Average.....		4.5	4.3	8.9	3.3

\* The difference between the circumference of the intact aorta and that of the isolated elastic ring is due to spontaneous retraction at zero load during extraction. The failure of the intact aorta and the isolated system to retract after near maximum extension is represented by percentages in the two right hand columns.

all external mechanical forces are removed. It is probable that the constraints are collagenous and that they operate in all axes of the intact aortic wall. A special study of the relationship between the magnitude of spontaneous retraction of elastic networks during purification and the elongation, dilatation and tortuosity of aged arterial channels is indicated.

The retraction of the intact aorta after near maximum mechanical extension is never sufficient to restore the vascular ring to its initial circumference. The degrees of incomplete retraction are recorded in

column 5 of table 1. The average is 8.9 per cent of the original circumference.

The retraction of isolated networks after near maximum extension is always greater than that of the aortic walls from which the networks were obtained. The values are recorded in column 6 of table 1. The average permanent elongation is 3.3 per cent of the final resting circumference of isolated networks. With due allowance for mechanical distortion incidental to application of breaking loads, this figure may be accepted as evidence of nearly perfect elasticity within limits of extensibility.

#### MEASUREMENTS OF BREAKING LOADS

Intact vascular rings will support very heavy loads. Breaking loads were not measured.

Measurements were made of loads which ruptured elastic rings after twenty-four, forty-eight and seventy-two hours of purification. The experimental values are recorded in the three right hand columns of table 2. There are great variations among these values due to variations in purity, dimensions and fragility of the elastic networks.

The average breaking loads after twenty-four, forty-eight and seventy-two hours' extraction of aortic walls are 112.4, 83.8 and 76.6 Gm., respectively. These differences indicate that in the average segment other components than elastic tissue contribute to the tensile strength at the end of twenty-four hours of extraction. The contribution of extraelastic components to the total strength diminishes in the twenty-four to forty-eight hour interval and is reduced to a negligible value after forty-eight to seventy-two hours of extraction. In this interval and in intervals after seventy-two hours the reduction in the average value for the breaking load is attributed to reduction in the tensile strength of elastic networks. This reduction is about twice as great as the value predicted by knowledge of the approximate quantity of elastic tissue dissolved by the reagent used for purification. In other words, from the physical point of view elastic tissue is degraded more rapidly than it is dissolved.

There are several conspicuous exceptions to the average behavior. Further studies will be necessary before an explanation that will comprehend all exceptions can be given.

#### DETERMINATIONS OF THE TENSILE STRENGTH OF ISOLATED NETWORKS

Methods for the estimation of tensile strength have been described.<sup>2</sup> Values for the tensile strength of elastic networks derived from 21 of the 34 aortas in this series were calculated. The results pertain to the tensile strength of networks after seventy-two hours of extraction and



are expressed in grams per square centimeter of cross-sectional area of elastic tissue reduced to dryness under the tension of a breaking load.

Values for the tensile strength of 13 of the 34 aortas were estimated by approximate methods. All values except the percentages of elastic

TABLE 2.—*Relations Between the Morphologic Changes in the Aortic Wall and the Changes in the Values for Near Maximum Extension and Breaking Loads During Purification of the Elastic Networks*

No.	Age, Yr.	Morphologic Findings			Extension After Given Number of Hours' Extraction, per Cent				Breaking Load After Given Number of Hours' Extraction, Gm.		
		Intima		Media Collagen							
		Lipids	Collagen		0	24	48	72	24	48	72
50	0	0	++	++	54.6	88.0	83.7	81.9	80	80	20
24	.08	0	++	++	41.4	90.8	89.8	87.9	80	50	30
44	.06	0	++	++	30.3	86.3	76.3	60.0	80	50	30
54	.06	0	++	++	41.2	77.1	69.2	77.1	60	40	35
35	.80	0	++	++	57.7	74.4	87.3	108.6	70	70	80
23	2.5	0	++	++	45.2	57.1	50.0	60.0	90	80	65
38	12	0	++	++	74.1	56.4	61.0	54.7	90	100	70
28	23	0	+	+	62.0	72.2	68.3	75.0	100	90	90
50	24	+	+	+	76.9	77.2	68.7	60.3	120	90	70
55	28	+	+	+	48.0	71.3	65.3	71.8	140	110	110
42	35	+++	+++	++	68.8	55.4	49.0	57.1	90	80	90
41	41	++	++	++	51.2	57.0	41.6	51.7	110	70	80
52	42	+	++	+	55.1	71.9	73.3	67.5	180	160	120
34	44	+++	++	++	59.8	66.7	57.4	56.0	130	90	70
39	45	++	++++	++	55.2	61.1	41.6	74.6	120	60	140
53	50	+	++	++	58.4	76.7	66.1	52.2	220	140	80
47	51	+	++	+++	55.4	57.8	61.0	63.1	110	110	100
36	51	+++	++	++	55.7	64.5	45.7	62.9	130	70	110
45	53	+	++	++	52.2	61.4	54.8	61.8	140	90	110
26	53	++	++++	++	42.8	70.6	70.3	62.2	200	110	70
43	54	++	+++++	+++	45.3	35.3	34.6	42.5	50	50	50
27	54	++	+++	+++	55.0	76.2	67.3	57.0	220	100	80
37	54	++	+++	+++	49.3	55.5	40.3	51.5	100	80	70
51	58	+	++	++	37.7	48.6	56.8	62.3	100	110	110
40	58	++	+++	+++	49.2	49.0	46.6	44.8	70	60	50
32	62	++	+++	++	41.2	50.2	59.1	60.4	70	80	80
48	62	++	++	+++	42.0	40.3	53.6	42.5	80	100	70
25	63	+	++	+++	38.0	77.2	60.5	60.0	170	80	50
33	67	+++	++	++	40.9	82.6	60.4	60.4	70	80	80
31	70	++++	++++	+++	29.7	58.9	53.6	70.0	90	80	110
46	71	++++	++++	+++	38.1	44.7	41.2	49.9	80	70	80
30	73	++	+++	++	38.4	79.2	71.2	80.4	140	110	110
29	74	+++	++++	+++++	38.8	66.1	43.4	40.0	80	40	30
49	77	++++	++++	++++	33.6	53.1	57.5	39.1	100	100	50
Average.....					48.9	65.0	59.7	61.9	112.4	83.8	76.6

tissue were known. The average percentage, obtained by a study of 21 aortas, was substituted for the unknown values in the formula for the estimation of tensile strength. The maximum error accompanying this substitution is no greater than 25 per cent.

The tensile strength of the elastic tissue of each aorta is recorded in table 4. The values range from 1,170 to 6,750 Gm., with an average of 3,700 Gm., per square centimeter.

## THE MORPHOLOGIC DATA

At least 4 vascular rings from each aorta were studied microscopically. One was a representative intact cylinder of the thoracic aorta. The remainder were similar control rings, extracted for twenty-four, forty-eight and seventy-two hours. They were fixed for twenty-four hours in Zenker's solution. The sections were stained by Mallory's aniline blue-orange G technic for demonstrating collagen and by the Weigert-Van Gieson method for staining elastic tissue.

The morphologic findings in the intact vascular segments were classified and graded in relation to the intima and the media. Estimates were made of the amounts of collagen and lipid in the intima. Any increase in collagen or lipid in the intima was always accompanied by an unestimated variable increase in elastic tissue.

The microscopic study of the media was restricted largely to an evaluation of increments in collagen and changes in structure of elastic tissue. In order to facilitate comparisons, the modifications of collagen were divided into three types. One type was a diffuse increase in collagen. A second was a focal increase in collagen. A third was a globular form of material which may not be collagenous.

The diffuse increase in collagen between elastic lamellae of the media is a well recognized morphologic change and in general becomes more conspicuous with advancing age.

The focal increase in collagen in the media has not, to my knowledge, been described. This is first observed in early life. The first evidence of the increase is a local condensation of bundles of delicate fibrils. These condensations occur in pairs, one on each surface of an elastic lamella. For this reason I have designated them as collagenous splints. They are roughly triangular in outline, the base of the triangle adjoining the elastic lamella and the apex projecting into the interlamellar region. They are usually larger and more conspicuous in adult than in young aortas. They reach maximum size in middle age and often are so compact that they seem to be homogeneous. They stain as does collagen with aniline blue, but not infrequently they also lightly accept the resorcinol-fuchsin elastic tissue stain.

The material classified as globular collagen is present in a few aortas. It has the same spatial distribution as the collagenous splints. It is possible to trace all stages of transformation of the collagenous splints into globular material. The globules, which vary from invisibility to about 10 microns in diameter, occur in aggregates. They possess some staining affinities of collagen but lack distinct fibrillar structure.

There are two significant morphologic changes in the elastic tissue of the media. The first change is the presence of structures which resemble crystals in the substance of elastic lamellae. The second change

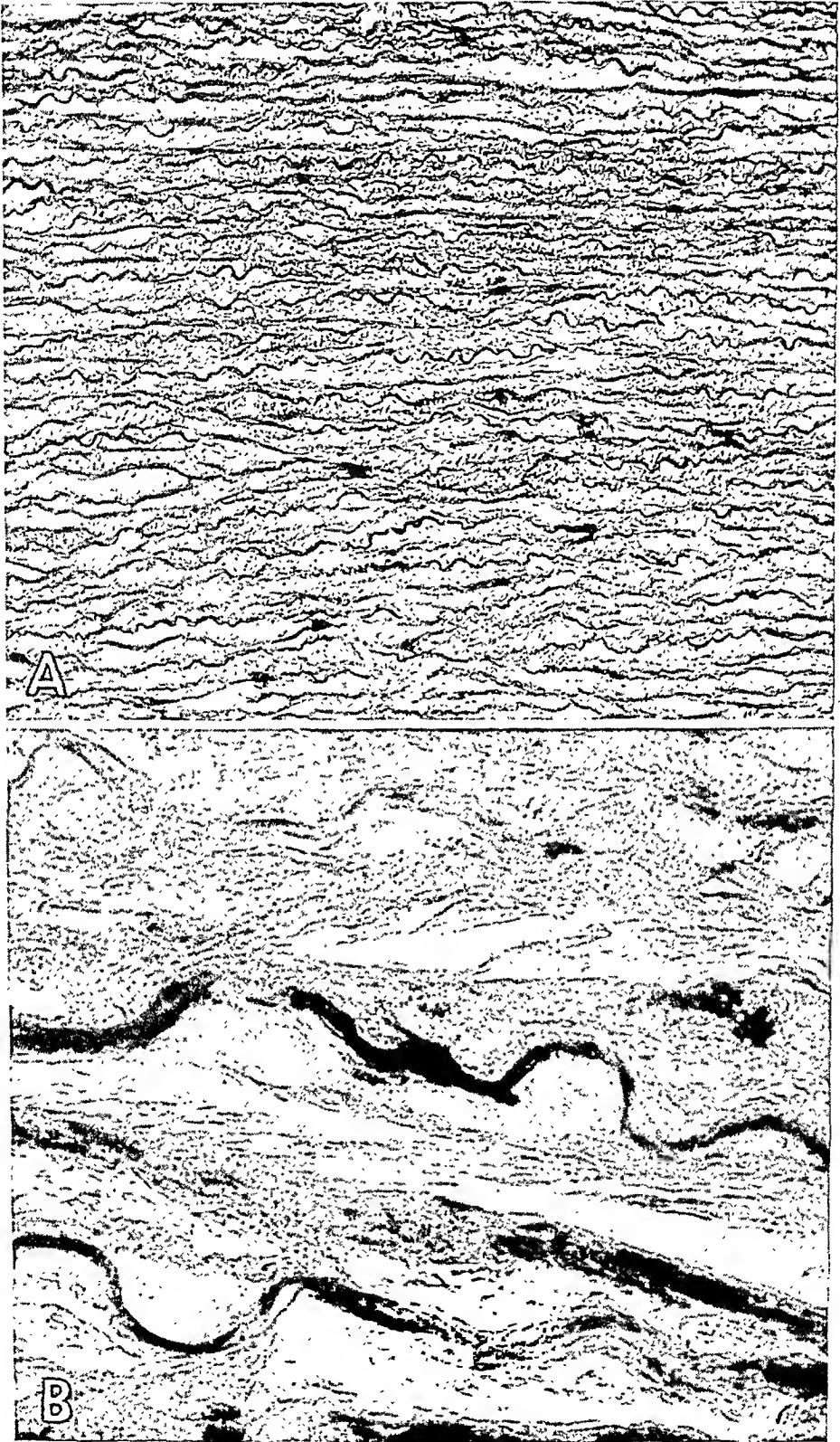


Figure 5

(See legend on opposite page)

is an accentuated discontinuity with atrophy of elastic lamellae. These findings are evaluated in a rough quantitative way, as shown in table 4.

The structures which resemble crystals are found in the aortas of a few middle-aged adults. With increasing age they are found less frequently and apparently are not present in aortas over 70 years of age. They occur in the axes of elastic lamellae at points where collagenous splints are found. Many are very small and are embedded in the elastin. Others are long narrow fusiform structures which occupy the axis of the elastic lamella for distances of 20 to 50 microns. They present transverse striations and are often divided into a chain of rectangular structures. They are unstained in the usual preparations, but there is often an accentuation of the depth of staining of elastic tissue in the immediate vicinity of the crystals. These features of form, distribution and structure are illustrated in figure 5. The crystals are highly refractile and are birefringent when viewed through crossed Nicol prisms. They are insoluble in fat solvents and resistant to the action of acids. Because of their optical properties, they are designated as axial crystals, although it is realized that there is no proof that they are either crystalline or even naturally occurring.

Discontinuity of elastic lamellae with variable degrees of atrophy is not conspicuous before the sixth decade of life. The discontinuities before the sixth decade of life are usually located at points occupied by axial crystals or splinted by bundles of collagen. Under these circumstances they are small. When they are large, no relationships to crystals or collagenous splints can be demonstrated. With the appearance of numerous broad discontinuities in elastic membranes, the lamellae often show atrophic changes. Collagen occupies the fenestrations in the membranes and diffusely increases throughout the interlamellar spaces. This morphologic picture is most conspicuous in aged aortas of low tensile strength.

#### CORRELATION OF MORPHOLOGIC PICTURE AND AGE

The changes in the intima in this series of aortas are the usual changes encountered with advancing age.

The first change in the media with increasing age is the appearance of juxta-lamellar collagenous splints. These are fairly numerous in the

#### EXPLANATION OF FIGURE 5

*A*, Weigert-Van Gieson preparation of an aorta. Several dark irregular areas in the substance of numerous elastic lamellae are shown. At each of these points the axial crystalline change described in the text and illustrated in *B* is found.

*B*, high power photomicrograph of one of the dark intralamellar areas shown in *A*. This sharply demarcated area interrupts the normal continuity of the elastic lamella. The optical properties of the material indicate that it is crystalline, and its solubilities indicate that it is neither a deposit of lipid nor one of calcium.

aorta of the 12 year old child. They are present in variable numbers in all older aortas, being more conspicuous in middle age than in the eighth decade of life.

The globular collagenous juxtalumellar masses have about the same topographic distribution as the collagenous splints, but they occur with less regularity and in older aortas.

The diffuse increase in collagen throughout the media does not become conspicuous prior to the sixth decade. The 2 oldest aortas present the best examples of diffuse fibrosis.

Discontinuity with local atrophy of elastic lamellae is a prominent change in a few aortas of advanced age. It is not a necessary accompaniment of age since no significant discontinuities were found in the lamellae of an aorta 73 years of age.

Axial crystals in elastic membranes have a peculiar age incidence. The youngest aorta in which they were found was that of a man 35 years of age. Crystals were found in 7 of 23 older aortas. As a rule, the older the aorta the less conspicuous were the crystals. No crystals were found in the 7 oldest aortas. As a rule, the crystals were accompanied by collagenous splints.

In brief, therefore, axial crystals, when they occur, appear only in elastic lamellae of middle-aged people. They diminish in frequency as diffuse medial fibrosis with discontinuity of elastic lamellae becomes increasingly conspicuous. In occasional aortas a spatial and temporal coincidence of axial crystals and discontinuity of lamellae is demonstrable. The nature and genesis of the spatially related collagenous splints, crystal patterns and discontinuities are not clear. Incomplete studies indicate that the crystal patterns, at least, are most numerous in aortas from patients with severe hypertensive cardiovascular disease.

#### CORRELATIONS OF MORPHOLOGIC PICTURE, AGE AND EXTENSIBILITY OF ELASTIC SYSTEMS

The intact vessel has low extensibility in infancy and in old age. The limitation of extensibility in old age increases in rough proportion to the increase of collagen in the intima and media.

The intact vessel is most extensible in the second and third decades of life. It is at about this stage of life that collagenous juxtalumellar splints acquire prominence. At a slightly older age the axial crystals in elastic lamellae often make their appearance in relation to the collagenous splints.

The isolated elastic networks display greatest extensibility in infancy. As a rule, extensibility is reduced with increasing age. The magnitude of this reduction in individual cases cannot be predicted from a knowledge of the morphologic appearance or extensibility of the intact aorta

from which the networks were isolated. For instance, the elastic systems isolated from 2 vessels, aged 70 and 73 years, possessed extensibilities of the same high order as infantile elastic systems. This became evident only after removal of constraints which restricted the extensibility of the networks in the intact vascular walls.

In contrast to this behavior of certain elastic systems after removal of constraints, there were several isolated systems which had less extensibility than the intact vessels. The best examples are those at 12, 24, 35, 44 and 50 years of age. All of these vessels had conspicuous collagenous splints, and 2 of them had numerous axial crystals.

TABLE 3.—*Relations Between the Morphologic Changes in the Aortic Wall, the Percentage of Elastic Tissue, the Minimum Cross-Sectional Area of the Isolated Elastic Tissue and the Tensile Strength of the Elastic Tissue*

Number	Age, Yr.	Morphologic Changes			Elastic Tissue, per Cent	Cross Section, Sq. Cm.	Tensile Strength of Elastic Tissue, Gm. per Sq. Cm.
		Intima		Media Collagen			
		Lipids	Collagen				
24	.03	0	±	±	28.9	0.0058	4870
38	12	0	±	±	40.6	0.0108	5040
55	28	+	+	+	41.1	0.0140	6750
42	35	+++	+++	++	40.2	0.0225	3140
41	41	++	++	++	40.4	0.0181	3360
52	42	+	++	+	40.9	0.0184	5450
34	44	+++	++	++	30.7	0.0229	2400
39	45	++	++++	++	34.2	0.0197	6220
53	50	+	++	++	38.2	0.0174	3490
47	51	+	++	+++	40.2	0.0206	3950
26	53	++	+++	++	37.4	0.0218	2600
45	53	+	++	++	36.3	0.0175	5090
27	54	++	+++	+++	38.5	0.0186	3380
43	54	++	++++	+++	42.2	0.0240	1490
40	58	++	+++	+++	30.1	0.0181	2000
51	58	+	++	++	41.9	0.0236	3790
48	62	++	++	+++	41.7	0.0253	1970
25	63	+	++	++	40.7	0.0226	1770
31	70	++++	+++	+++	41.5	0.0270	3450
46	71	++++	++++	++++	31.1	0.0165	3490
49	77	++++	+++	+++	39.0	0.0234	1490

Despite great variations in the magnitude of extensibility of different networks, the shapes of the extensibility curves were similar. The differences in the slopes are more apparent than real because all the curves are reduced nearly to a common slope when values are adjusted to correct for differences in cross-sectional areas of various isolated elastic rings. Hence, within the limits of variable fragility the usual response of the elastic system to an applied load is independent of age or morphologic change.

#### CORRELATIONS OF MORPHOLOGIC PICTURE, AGE, EXTENSIBILITY AND RETRACTILITY

No consistent relationships between morphologic appearance, age, extensibility and retractility of intact vascular walls were found. The impression was gained that measurements of speed of retraction might reveal some interesting correlations.

On release of loads producing near maximum extension the retractility of isolated networks was always greater than that of the corresponding intact vascular walls. On release of loads producing half maximum extension the isolated networks exhibited, for practical purposes, perfect elastic retraction. The data in tables 1 and 4 show the similarity of retractile powers of different networks after near maximum extension and the lack of any influence of age or morphologic change on the capacity to retract.

One type of retraction does depend on age and morphologic change. This is the spontaneous retraction of a few networks at zero load after extraction of nonelastic components of the vascular wall. As shown in tables 1 and 4, this behavior is encountered especially among aged dilated aortas of low extensibility. It seems that the networks in these vessels are maintained in a state of slight extension by dense collagen that passes between the elastic lamellae and through the discontinuities in the membranes.

#### CORRELATIONS OF MORPHOLOGIC APPEARANCE, AGE, EXTENSIBILITY, RETRACTILITY AND TENSILE STRENGTH

As a rule, isolated elastic systems of average or greater than average tensile strength possessed an average or greater than average extensibility. This relationship was not quantitative. Presumably, lamellae of high quality and continuity persisting alongside networks of poor quality or discontinuous structure are responsible for the absence of a better quantitative relationship.

The degree of retraction of isolated networks after near maximum extension was similar for all networks and was therefore independent of tensile strength.

The tensile strength of infantile elastic systems was not as great as that predicted from morphologic studies. However, the average value exceeded the average value for the entire series. A more precise method for measuring tensile strength should be developed before data with respect to the delicate aortic rings of infancy are accepted as having quantitative significance. I do not believe that the estimates shown in the last column of table 4 are close to the true values.

In early adult life the isolated elastic networks, with occasional important exceptions, have a high tensile strength.

In the late decades of life the tensile strength of isolated networks, with but one exception, falls to a low value. The lowest value, 1,170 Gm. per square centimeter, at 74 years of age, is to be compared with the maximum value, 6,750 Gm. per square centimeter, at 28 years of age.

From infancy until the fourth decade of life the only significant morphologic change is the appearance of collagenous splints in the

media. The observed number of splints is not specifically related to the tensile strength of isolated networks.

Nine aortas between the ages of 28 and 51 were studied. Five had networks with tensile strengths of 4,000 to 6,750 Gm. Four networks had tensile strengths varying from 2,400 to 3,500 Gm. Of the 4 vessels with low tensile strength, 3 had a large number of axial crystals in the elastic lamellae and a corresponding number of associated collagenous

TABLE 4.—*Relations Between the Morphologic Changes in the Media of the Aortic Wall and the Tensile Strength of the Isolated Elastic Networks*

No.	Age, Yr.	Morphologic Changes in Media				Tensile Strength, Gm. per Sq. Cm.	
		Collagen			Elastic Tissue		
		Diffuse	Globular	Splint	Intrinsic Crystals		Atrophy and Discontinuity
50	0	++	0	0	0		3600 (est)
24	.03	+++	0	0	0	.....	4870
44	.06	+++	0	0	0	.....	3000 (est)
54	.06	+++	0	0	0	.....	5100 (est)
35	.80	+++	0	0	0	.....	5830 (est)
23	2.5	+++	0	0	0	.....	3600 (est)
35	12	++	+	++	0	.....	5040
28	23	+	0	+++	0	.....	6600 (est)
56	24	+	0	+	0	.....	3240 (est)
55	28	+	0	++	0	.....	6750
42	35	++	0	++++	++++	.....	3140
41	41	++	+	+++	+++	.....	3360
52	42	+	0	++	+	.....	5450
34	44	++	0	++++	++++	+	2400
39	45	++	+++	+	0	.....	6220
53	50	++	+	++	+	+	3490
47	51	+++	+	+	0	+	3950
36	51	++	++++	++++	0	.....	5000 (est)
45	53	++	0	++	0	.....	5090
26	53	++	0	+++	0	+++	2600
43	54	+++	0	++++	+++	+	1490
27	54	++++	0	+	0	+++	3380
37	54	+++	+++	+++	0	++	2680 (est)
51	58	++	+	+++	+++	+	3790
40	58	+++	+++	++	0	+++	2000
32	62	++	0	+++	0	++	2580 (est)
48	62	+++	0	+++	+++	+++	1970
25	63	+++	0	++	0	+++	1770
33	67	++	+++	+++	0	++	2860 (est)
31	70	+++	0	++++	0	++	3450
46	71	+++	++	++	0	+	3490
30	73	++	+++	+	0	+	5470 (est)
29	74	+++++	0	++	0	++++	1170 (est)
49	77	++++	+	++	0	+++	1490

splints. One vessel with high tensile strength had rare axial crystals, and another vessel with high tensile strength had numerous collagenous splints. Other morphologic changes in the intima and media were not conspicuous, though the usual average increase in collagen that is expected in this age interval was encountered. It is possible to conclude from these data that the presence of axial crystals in elastic lamellae is the first morphologic evidence of reduced tensile strength of elastic systems in early middle life.

In the late decades of life there is an average progressive reduction in tensile strength. The morphologic counterparts of this reduction are



increase in collagen in the intima, increase in diffuse collagen in the media, increase in discontinuity with atrophy of elastic lamella, decrease in the number of collagenous splints and decrease to zero of axial crystals at advanced age. Among the positive findings, discontinuity of elastic lamellae is the morphologic change which is most closely related to reduction in tensile strength of the aged aortic elastic tissue.

#### A CONCEPT OF SEQUENCES IN ATHEROSCLEROTIC VASCULAR DISEASE

In general, it is possible to account for most findings in the case of atherosclerosis if it is assumed that there has been prolonged imbalance between the tension on the walls of vascular structures and the capacity of these structures to compensate for the imbalance. The imbalance may be a consequence of changes in blood pressure or of changes in the wall of the vessel or of both. Observations indicate that in general the imbalance is due to an elevation of intravascular pressure whether this is in the peripheral arterial system, in the pulmonary arteriovenous system or in the portal venous system, and that in the absence of other compensating forces the result is atherosclerosis. It is clear that the elastic systems, especially those in highly extensible vessels, must bear the brunt of the uncompensated elevation of tension.

According to the present studies, the elastic systems of the infantile aorta are in a position to withstand undue elevations of internal pressure because they are protected against overdistention and cannot be brought to their maximum extensibility by application of very large loads. There is a protected reserve. By the end of the second decade of life there is no protected reserve. By application of moderate tension the elastic networks in the wall of the intact vessel become extended to or beyond the maximum attainable by isolated networks. Hence, in the early decades of middle life the elastic system of the aorta is most susceptible to injury by an increase of tension on the vascular wall. It is during this period of life that collagenous juxta-lamellar splints appear, and at a slightly older age, in the fourth and fifth decades, the elastic lamellae between these splints often show a peculiar form of axial crystallization. This is the earliest morphologic sign of a reduction in the tensile strength of networks. In the course of time the lamellae undergo local disintegration at foci of axial crystallization. The membranes develop broad discontinuities which become filled with collagen that increases generally in the interlamellar spaces. Though some membranes remain intact, the tensile strength of the elastic systems is reduced to a low level. Distention and elongation of the vascular walls occur with collagenous fixation of the residual networks in a state of moderate extension. Extraction of the collagen permits retraction of these networks to near normal dimensions. Once free from

constraints, they display, irrespective of age, elastic properties which within the limits of extensibility are identical with those of infantile elastic tissue.

The concept which has been presented accounts for most coincidences of physical measurements and morphologic findings. It fails, perhaps, to place correct emphasis on the morphologic changes in the intima. The possible failure is due to the judgment that the intimal changes represent a fortuitous accumulation of lipids in a collagenous splint that is deposited in response to a primary failure of medial systems to maintain integrity of function in the presence of imposed tensions.

#### SUMMARY

It is not possible to predict the mechanical or dynamic behavior of isolated elastic networks from knowledge of the behavior of the aortic walls from which they are obtained. The average purified networks are more extensible than the intact aorta. The extensibility is greatest among young networks and usually decreases with age, but occasional aged elastic systems have the characteristic high extensibility of youthful tissue.

The low extensibility of aged dilated intact aortas is partly due to the fact that at zero load the elastic networks are under tension. When the constraints responsible for this constant tension are removed, the elastic networks spontaneously retract to the dimensions of an undilated aorta.

After removal of mechanical or chemical forces, either of which may produce near maximum extension, all purified elastic systems, irrespective of age, exhibit identical and almost perfect retraction.

The tensile strength of isolated networks is, as a rule, high in the early decades and low in the late decades of life. When elastic systems with low tensile strength are encountered in middle life, peculiar crystal patterns abutted by collagenous splints are found in the axes of elastic lamellae. Elastic systems with low tensile strength in late life have conspicuous discontinuities but no axial crystals. The evidence indicates that some of these discontinuities arise by disintegration of elastic lamellae at focal points of collagenous splinting and axial crystallization. The genesis of these medial changes and their contribution to the formation of atherosclerotic lesions remain obscure.

# ADRENAL RESTS IN THE KIDNEY

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As late as 1940 the statement "adrenal rests occur so infrequently in the kidneys that they seem relatively unimportant in explaining the development of cancer of the kidney in adults" appeared in the literature.<sup>1</sup> It is the purpose of this presentation to refute that statement by describing 23 instances of adrenal rests in the kidneys, encountered in 2,896 autopsies in a six year period at the Queens General Hospital. Nelson<sup>2</sup> adequately reviewed the reported cases of aberrant adrenal tissue in various sites. Of those authors who have described studies of adrenal rests in kidneys he cited Lubarsch, and Glynn and Brites. Lubarsch found adrenal cortical tissue in 8 kidneys of 300 autopsies. Brites listed 10 cases of aberrant adrenal tissue in a series of 376 cases in which the kidneys were examined. Glynn, and Brites in a later study, found no instance of adrenal cortical tissue in 1,500 cases. In a group of 630 autopsies by Nelson, 5 instances of accessory adrenal tissue under the renal capsule were encountered.

Excluded from this study are those cases of adrenal heterotopia described by Weller,<sup>3</sup> and duplication of the adrenal. The cases listed in the accompanying table are those in which microscopic sections have substantiated the gross diagnosis of ectopic adrenal tissue.

## INCIDENCE

It may be stated at this juncture that the true incidence of adrenal rests in kidneys is a direct function of the care which is taken in the dissection of the kidneys during routine autopsy procedures. To illustrate this point, the six year period of this study may be divided into two parts. Only during the latter two and one-half years was a con-

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1. Caspar, I. A.: New York State J. Med. **40**:1209, 1940.

2. Nelson, A. A.: Arch. Path. **27**:955, 1939.

3. Weller, C. V.: Am. J. M. Sc. **169**:696, 1925.

scious effort made to note the presence of adrenal rests and to establish the diagnosis with certainty in all such instances by taking sections for microscopic study. It should be emphasized further that the incidence cited in the table is undoubtedly not a true figure. Serial sections were not made, and many minute lesions were not confirmed microscopically because of inadequate histologic technic. Thus, while there have been recorded in this laboratory only 23 instances of adrenal rests out of a total of 2,896 autopsies, fully 22 have been observed during the last 1,806 autopsies from July 1, 1939 to Jan. 1, 1942. During the latter interval, the renal capsule of each of the two kidneys was carefully stripped away from the underlying cortex, so that every millimeter of cortex was examined. Every suspicious nodule of tissue that appeared to be different from the regional renal cortex was studied histologically.

The table indicates the distribution of the recorded cases by decades of life.

*Distribution by Decades*

Decade	Cases	Total Number of Cases in Autopsy Series
1.....	2	522
2.....	1	109
3.....	0	202
4.....	2	256
5.....	4	403
6.....	5	530
7.....	6	499
8.....	3	302
9.....	0	69
10.....	0	4
	23	2,896

The ages ranged from 1 year to 76 years, with a mean age of 42.3. The increase of incidence with advancing age is only apparent, for the neonatal group of cases was not studied as carefully grossly, and histologic confirmation was lacking in many instances.

GROSS APPEARANCE

The adrenal rests invariably appeared as bright yellow flattened nodules of soft semitranslucent tissue situated beneath the renal capsule (fig. 1 *A*) or adherent to its inner surface. In the majority of instances the nodule was found at or near the upper pole of the kidney. Occasionally one was seen near the midzone. The nodules were remarkably uniform in size, most measuring no more than 3 to 4 mm. in their

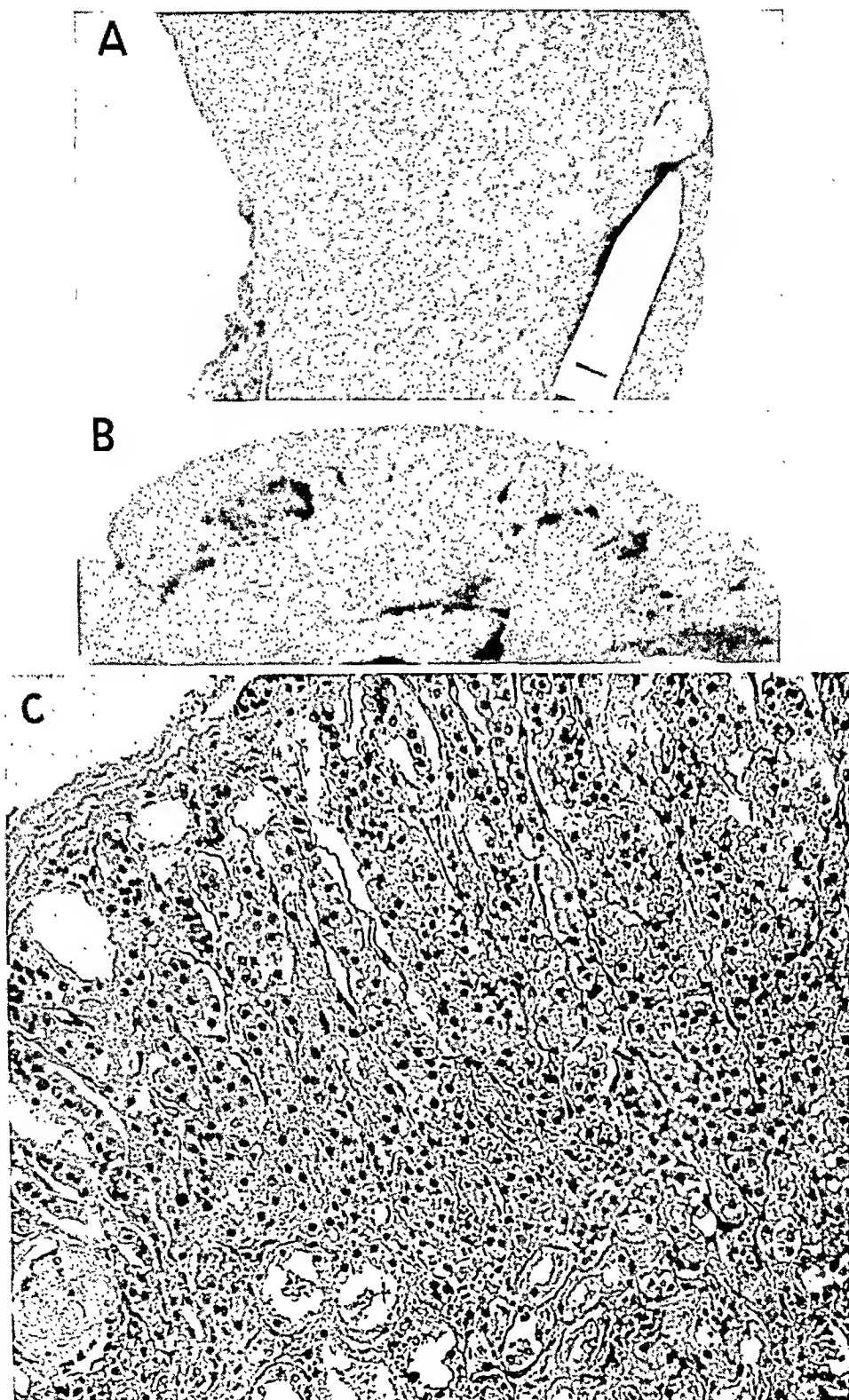


Fig. 1.—*A*, small flattened and sharply delimited adrenal in the midzone of a kidney. *B*, transected oval mass of ectopic adrenal showing centrally placed brownish area resembling zona reticularis, at extreme right of figure. *C*, subcapsular adrenal rest showing well developed zona glomerulosa and zona fasciculata without underlying delimiting capsule.

largest diameter. On section the nodules penetrated into the subjacent renal cortex for a distance of 0.5 to 1 mm. Usually a sharp line of demarcation was seen at this point. Some of the nodules revealed a central area of brownish discoloration which resembled zona reticularis (fig. 1 B). In a few instances the rest was stripped with the capsule. In others rest tissue remained on the capsule and on the kidney surface.

#### MICROSCOPIC APPEARANCE

The typical adrenal rest nodule presented itself as columns of adrenal cortical cells situated beneath the true renal capsule. The zona glomerulosa and fasciculata were almost invariably present (fig. 1 C), while a less common finding was that of cells resembling the zona reticularis. In none of the cases was adrenal medullary tissue found. Changes commonly observed in normally situated adrenal tissue, as focal and diffuse lipoidosis, were noted in the adrenal rests. Areas of liposis (fig. 2 A), cysts of microscopic size and marked congestion of the zona reticularis were seen on occasion. Some of the cysts might well represent included cystic, dilated renal tubular elements. Although the true renal capsule formed the outer covering of each of the nodules described, occasionally another partial capsule separated the adrenal cortical tissue from the underlying renal parenchyma over wide zones. It is significant that this inner capsule was invariably incompletely formed. In 1 case there was a rather marked irregular deep penetration of the adrenal tissue in among the renal tubules. The presence of papillary ingrowths in miniature cysts of undoubted adrenal origin is of considerable theoretic importance in the consideration of renal neoplasms (fig. 2 B).

Although it was not the original purpose of this presentation to enter into the oft-disputed histogenesis of hypernephroma, the occurrence of adrenal rests in 2 cases of hypernephroid tumors in the present series seems worthy of comment. In one there was a soft well circumscribed mass of yellowish tissue in the kidney, measuring 8 cm. in diameter, at the summit of which a small adrenal rest was noted (fig. 3 A). No regional extension or distant metastasis was noted in this case. Microscopically, this tumor was identical in structure to the separate ectopic rest and was of undoubted adrenal origin. A peculiar grouping of adrenal cortical cells arranged in a formation resembling a glomerulus was of interest (fig. 3 B). In the other case a small

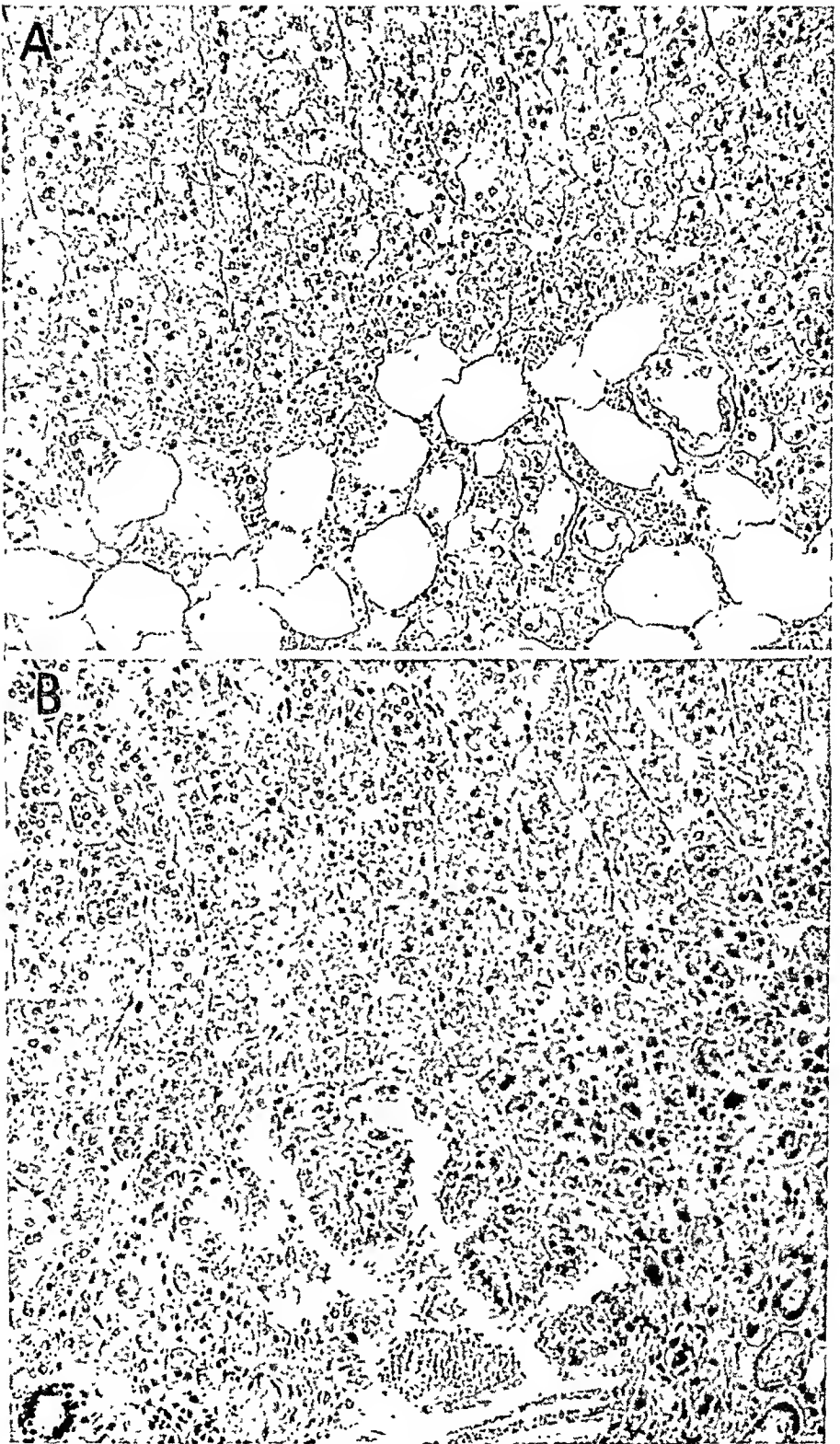


Fig. 2.—*A*, subcapsular adrenal rest showing true liposis at the junction with the kidney. The zona glomerulosa and the capsule are not included in the photograph. *B*, photograph showing zona fasciculata and zona reticularis in a subcapsular adrenal rest. The adjoining renal tubules are seen at the lower right. Note the papillation in the cyst of the adrenal cortical tissue. The zona fasciculata shows scant vacuolation.

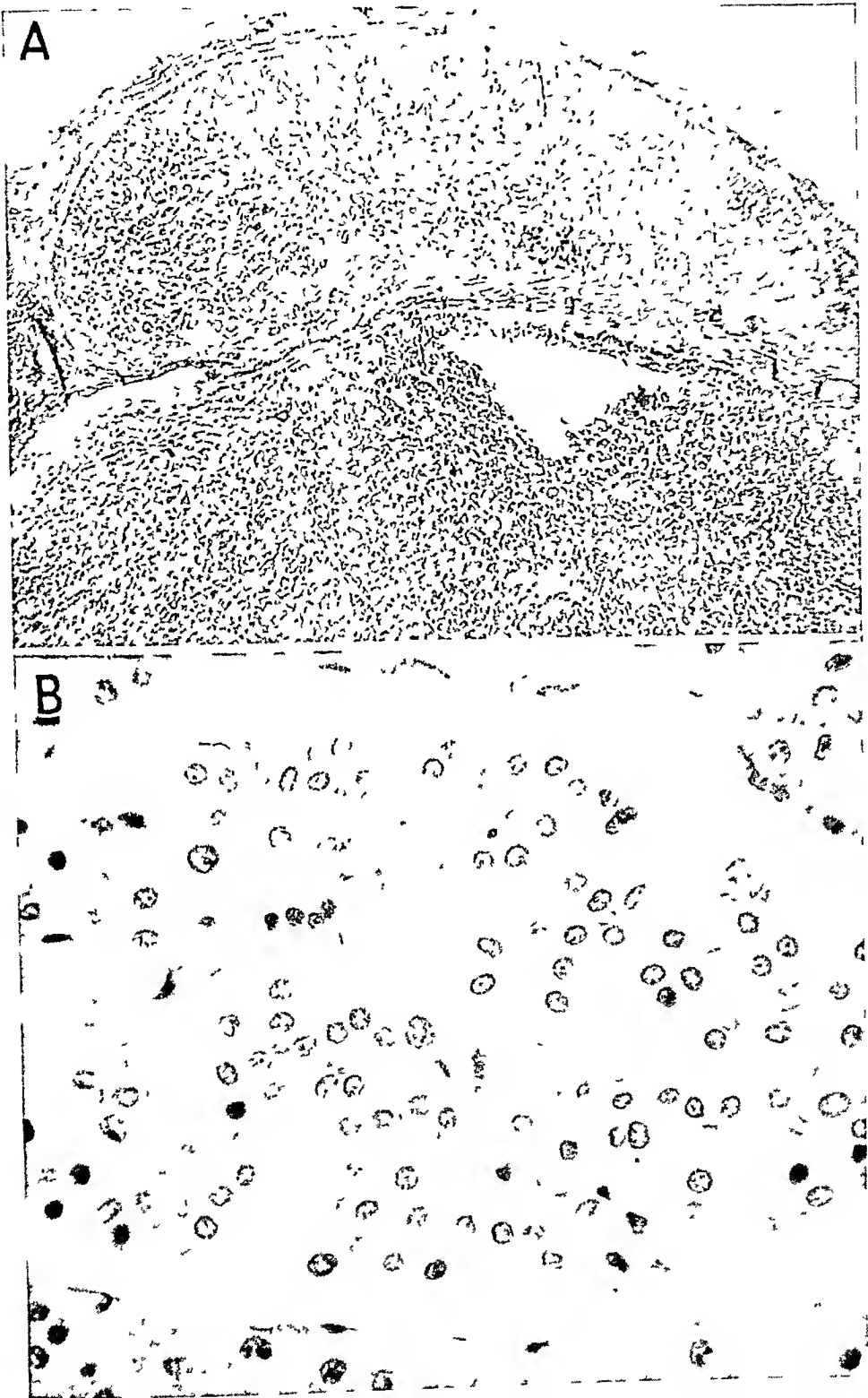


Fig. 3.—*A*, adrenal rest in a bifurcation of the capsule and an encapsulated adenomatous nodule of identical cytologic structure in the kidney substance. *B*, glomerulous-like structure composed of adrenal cortical cells, situated deep within a tumor of the kidney diagnosed as adrenal adenoma. (See *A*.)



adrenal rest was seen distinct from and above a cancer of the kidney (fig. 4). Widespread metastases of the hypernephroma were present in this instance. The finding of adrenal rests in association with these two renal tumors might well be a coincidence. However, the identity of the histologic picture, the tendency to extend down into the kidney, the gradation in size and proliferation of the nodules formed from miniature adenomatous growths to tumors diagnosed as hypernephroma add significance to such simultaneous occurrences.



Fig. 4.—Hypernephroma of the kidney with satellite extensions. An arrow points to a subcapsular adrenal rest at the superior pole.

#### SUMMARY

Adrenal rests occur commonly in kidneys. Twenty-three instances are reported.

If the renal capsule is stripped carefully and the exposed surface of the kidney and the capsule are minutely examined, the incidence of such foci increases markedly.

Reference is made to the theoretic significance of such foci in relation to the origin of hypernephroma.

# DEVELOPMENTAL BASIS OF REGENERATIVE AND PATHOLOGIC GROWTH IN THE UTERUS

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A series of investigations concerned with the embryonic development of the urogenital organs yielded results which may be of value for the interpretation of normal and pathologic structure of the uterovaginal canal. Some of these have been described in previous reports<sup>1</sup> and will be mentioned here only as far as necessary for the understanding of new observations and conclusions. The embryologic portion of the present report will be confined to observations which bear on the problem of the formative potentialities of the tissues concerned. A subsequent section will deal with the possible applications of these observations for the understanding of normal and pathologic changes, such as normal regeneration of the uterine epithelium, the structure of tumors of the uterovaginal canal and the formation of endometriosis.

## OBSERVATIONS

Serial sections of many human, mammalian and chick embryos were used in the present work. The principal staining methods employed were azan staining and silver impregnation of the reticular fibers. Findings in chick embryos will be described only when they facilitate the interpretation of observations in human embryos by showing clearer pictures of comparable conditions. The müllerian duct as the embryonic forerunner of the uterovaginal canal will be in the center of these discussions. However, the description of its development will be preceded by a section concerned with its mother tissue, the celomic wall.

*Early Organ Formation from the Celomic Wall.*—It has long been known that in the embryo at an early stage of its development the wall of the celomic cavity shows no epithelial surface lining distinctly separated from the underlying mesenchyme. The superficial cells have, of necessity, on the side facing the cavity an arrangement similar to an epithelium. On their basal side, however, these same cells have protoplasmic processes structurally identical with those of the adjacent mesenchymal cells (fig. 1 a), and cells resulting from division of the

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1. Gruenwald, P.: (a) *Anat. Rec.* **81**:1, 1941; (b) *J. Morphol.* **70**:353, 1942.

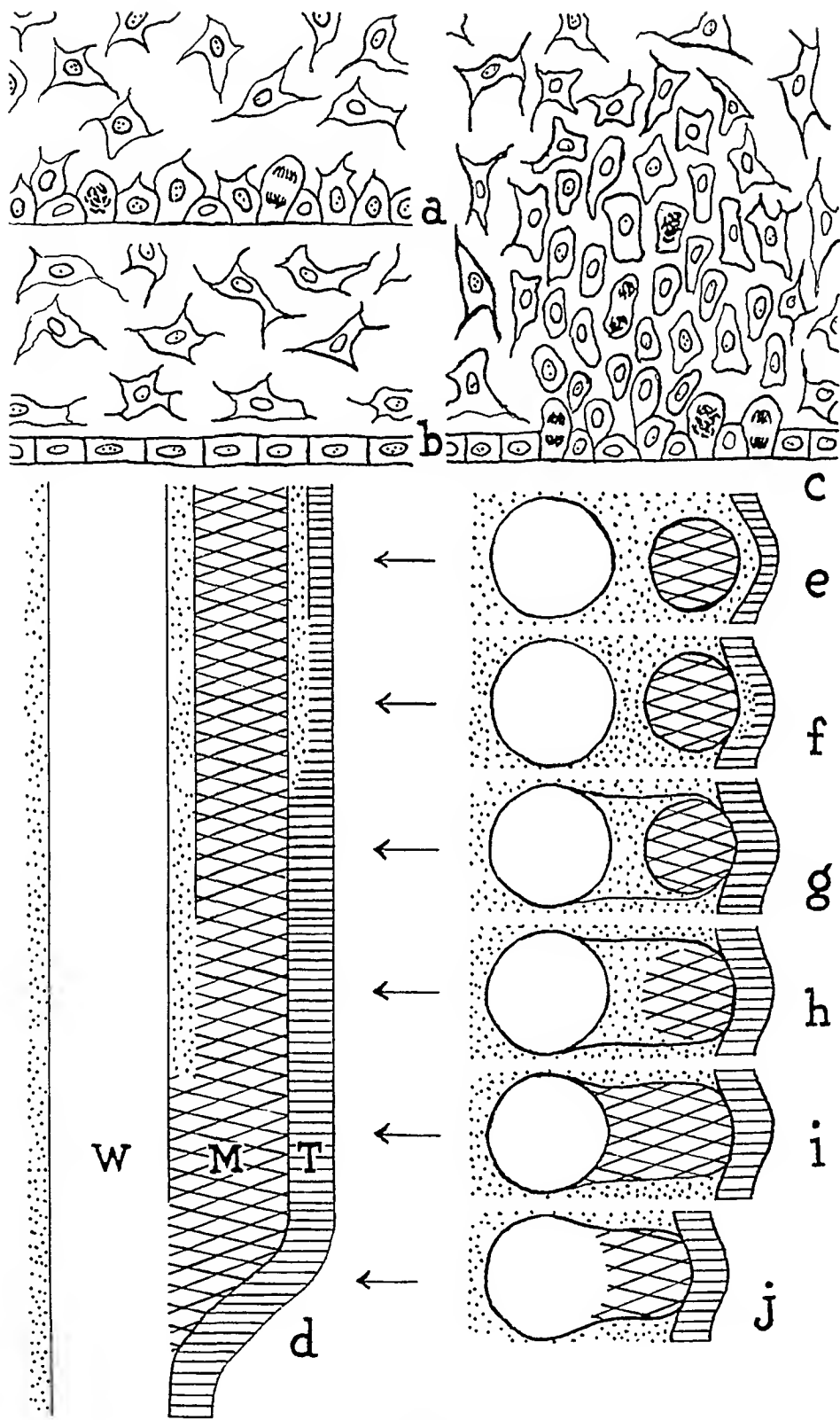


Figure 1

(See legend on opposite page)

superficial cells move into the mesenchyme to become part of it. This process has been described by numerous embryologists and extensively evaluated by Maximow.<sup>2</sup> The superficial cells soon differentiate into a typical epithelium clearly separated from the underlying mesenchyme (fig. 1 *b*). This transformation is almost complete in human embryos of about 8 mm. As pointed out previously in more detail,<sup>1b</sup> this change does not represent permanent and irrevocable specialization. Not only does the celomic wall in some areas revert to the early condition, thus allowing for contribution of cells from the superficial layer to the adjacent mesenchyme, but organs developing from the celomic wall retain in their tissues a tendency toward changes between epithelial and mesenchymal structure. In the celomic wall itself this becomes apparent when large numbers of cells are to be produced for the formation of an organ. In early development of the gonads, for instance, the earlier established boundary between celomic epithelium and mesenchyme disappears, and both parts of the celomic wall participate as a uniform tissue in the formation of the gonadal blastema.<sup>1b</sup> Similar changes may be observed during the formation of the adrenal cortex. A diagram of this type of organ formation from the celomic wall is given in figure 1 *c*. It should be mentioned at this point that a similar participation of the celomic lining in the formation of a blastema also occurs in the early stages of limb bud development. This was first shown in amphibians by Filatow.<sup>3</sup> The occurrence of a very similar condition in human embryos is described and its significance for the problem of endometriosis evaluated in a separate

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2. Maximow, A.: Arch. f. exper. Zellforsch. 4:1, 1927; in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, pt. 1, p. 232.

3. Filatow, D.: Arch. f. Entwicklungsmechn. d. Organ. 127:776, 1933.

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#### EXPLANATION OF FIGURE 1

Diagrams illustrating the formation of mesenchyme from the lining of the embryonic celomic wall and the müllerian duct. The early celomic wall is shown in parts *a* to *c*: In *a* one sees the early stage. No definite epithelium is present, and the superficial cell layer contributes to the deeper layers. In *b* a later stage is shown, after differentiation of the superficial layer into epithelium. In *c* is presented the condition during formation of an organ primordium. The early structure is reestablished and the surface lining contributes toward the organ blastema without forming epithelial buds. Parts *d* to *j* represent the müllerian duct and the tubal ridge: *d* shows a longitudinal section; *e* to *j*, cross sections at the levels indicated by arrows. See explanation in the text. *M* indicates the müllerian duct; *T*, the tubal ridge; *W*, the wolffian duct; the mesenchyme is stippled.

article.<sup>4</sup> In contrast to these structures, the müllerian ducts develop from the celomic wall not by a blastema but as epithelial growths from the celomic epithelium.

In the gonads and the adrenal cortices the lability of epithelial tissue structure is not limited to early stages of blastema formation. Numerous examples of transitions from epithelial to nonepithelial structure or vice versa may be seen in these organs in much later stages as well.<sup>1b</sup> As to the müllerian ducts, it appeared as if the epithelial character was definitely and permanently established from the earliest stages of development. However, it will soon be pointed out that transitions from epithelial to mesenchymal structure also occur in the tissue of the müllerian primordia, thus characterizing them as typical celomic derivatives along with the adrenal cortices and the gonads.

*Development of the Müllerian Ducts.*—The longitudinal growth of the müllerian ducts was previously studied in detail,<sup>1a</sup> and their extremely close topographic relations to the wolffian ducts were described at that time. As commonly known, the müllerian ducts arise from funnel-shaped invaginations of the celomic epithelium, the later ostia tubarum, and grow caudad alongside the wolffian ducts without any further contribution from the celomic epithelium. A continuation of the tall celomic epithelium of each ostium may be traced caudad on the surface of the urogenital ridge, covering the wolffian and later the müllerian duct. This strip of tall epithelium, also known as the tubal ridge, neither contributes to the müllerian primordium nor regenerates a müllerian duct if the development of the original duct is inhibited experimentally.<sup>5</sup> Its further development will be described in a later section of this report. The müllerian duct itself is found very intimately connected with the wolffian duct soon after it grows out from the ostium. Its growing end is seen as a solid wedge between the epithelium and the basal membrane of the wolffian duct, as previously <sup>1a</sup> illustrated by photographs and in the present figure 1 *d* and *j* by diagrams. As this wedge moves caudad, the müllerian duct lengthens and is gradually separated from the wolffian duct (fig. 1 *d* and *i*).

Evidence of the formation of mesenchyme from the müllerian primordium was first found in chick embryos and a cat embryo and was briefly described in connection with a review of the development of celomic derivatives in general.<sup>1b</sup> The observations in chick embryos have since been expanded by addition of new material, and comparable conditions have been found in human embryos. In the following account the findings in chick embryos will be reported first, because they are clearcut and easily interpreted. This will facilitate the understanding

4. Gruenwald, P.: Am. J. Obst. & Gynec. **44**:470, 1942.

5. Gruenwald, P.: Arch. f. Entwicklungsmechn. d. Organ. **136**:786, 1937.

of the less extensive changes in human embryos. The site of these changes is the latest formed caudal portion of the growing müllerian duct, whose relation to the wolffian duct has just been described. As does the growth of the duct itself, mesenchyme formation proceeds in a caudal direction so that all stages may be studied in one embryo by following the series of transverse sections in caudocranial succession. Evidence of mesenchyme formation was found only during the later period of growth of the müllerian duct within the caudal portion of the urogenital ridge; however, no distinct cranial boundary of this area can be given. Figure 2 *a-d* shows four stages of the process to be described, in sections of the left müllerian duct and surrounding structures of a chick embryo of 6 days 3 hours. The most caudal section (fig. 2 *a*) shows the solid growing end of the müllerian duct connected with the wolffian duct partly without separating basal membranes. Figure 2 *b*, taken from a section 110 microns cranial to the previous one, shows the wolffian duct separated from the müllerian primordium by a delicate but complete basal membrane. The müllerian primordium itself appears divided, consisting of the duct proper and a pair of wedges cutting in from the medial and lateral sides between it and the wolffian duct. These wedges, being part of the müllerian primordium and located inside the basal membrane, exhibit early stages of the appearance of argyrophil fibers between their cells, indicating transformation into embryonic connective tissue. Figure 2 *c*, showing a section 280 microns farther cranially, presents a more advanced stage. The müllerian duct proper, representing only part of the original primordium, has also received a basal membrane, while that of the original primordium begins to disappear (left side of fig. 2 *c*). The aforementioned medial and lateral wedges of müllerian tissue are now part of the mesenchyme surrounding the ducts. Somewhat later, after complete dissolution of the basal membrane of the original müllerian primordium, they cannot be distinguished from the mesenchyme previously present in that area. Figure 2 *d*, of a section 1,170 microns cranial to that of figure 2 *c*, shows a mass of mesenchyme surrounding the müllerian duct and bounded peripherally by a denser network of argyrophil fibers. It cannot be established whether this mass corresponds to the tissue given off by the müllerian primordium.

The earliest signs of mesenchyme formation from the müllerian primordium were found in an embryo 5 days 18 hours old, and the last remnants of the disintegrating basal membrane of the original müllerian cell mass are still visible in a 7 day embryo. The clearest pictures of mesenchyme formation can be observed in embryos of the early part of the seventh day. Figure 2 *e* shows a section of the right müllerian primordium of a 6 day embryo during the process of subdivision into

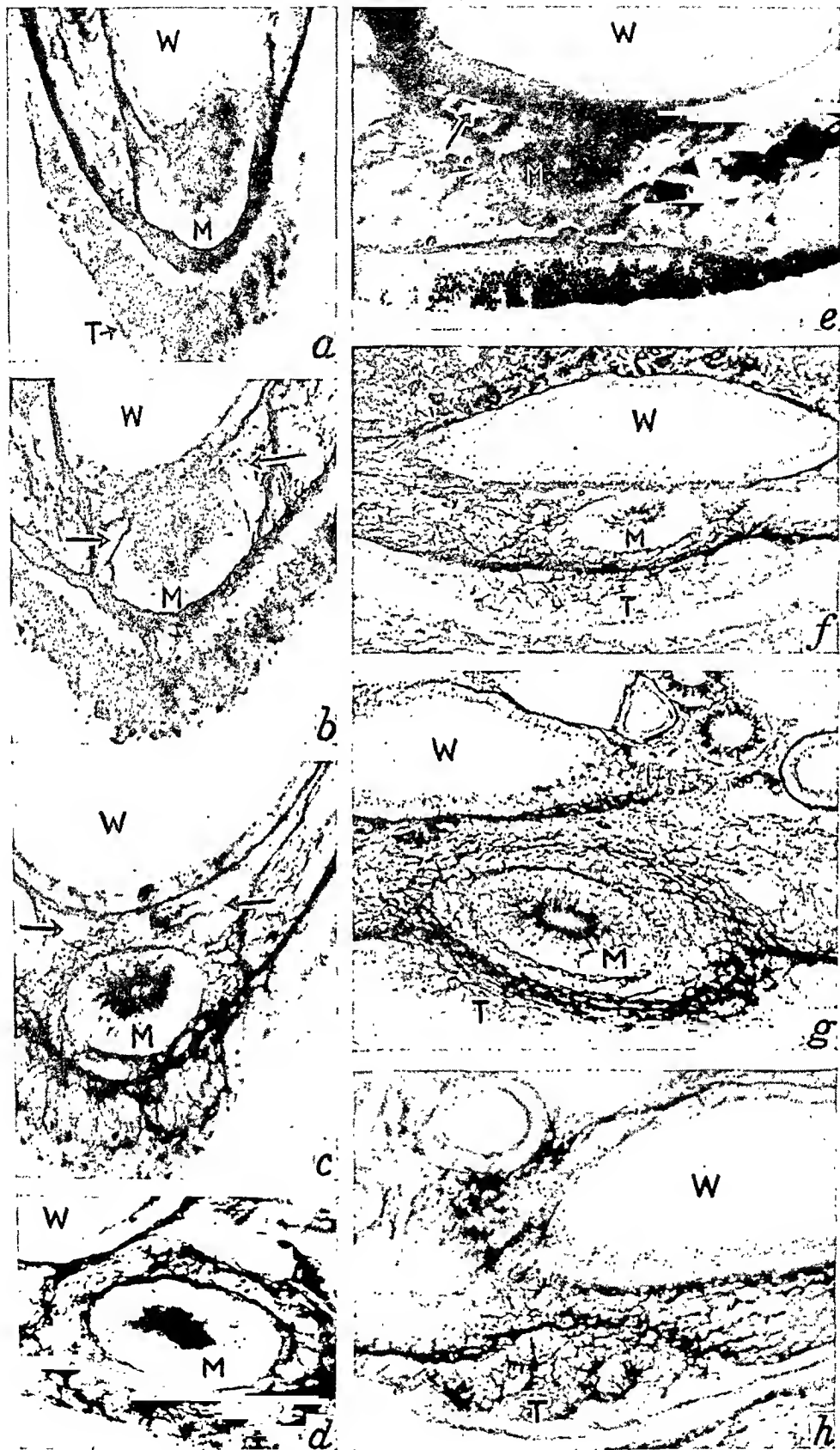


Figure 2

(See legend on opposite page)

the duct proper and mesenchyme. More cellular details are seen in this figure because the silver impregnation was supplemented by a counter-stain with acid fuchsin. A heavy common basal membrane surrounds the wolffian duct and the müllerian primordium; within the latter a subdivision into the duct itself and the loosely arranged cell groups of the wedges is clearly visible. In the absence of the acid fuchsin stain this loose tissue would show the beginning of fiber formation between its cells, similar to that seen in figure 2 *b*.

Similar changes may be seen in the müllerian primordia of mammal embryos. The only cat embryo (22 mm.) available for the present work showed evidence of large wedges of mesenchyme between the wolffian and müllerian ducts, within the common basal membrane (Gruenwald,<sup>1b</sup> fig. 19). In human embryos of from about 20 to 25 mm., silver impregnation reveals similar wedges (fig. 3 *a-c*). The pictures here are not as distinct as those of chick embryos but correspond in essential traits. The reason for the difference is not only the probably smaller amount of mesenchyme formed from the müllerian primordium in the human embryo but also the closer succession of stages. The latter difference is expressed in the detachment of the müllerian from the wolffian duct as well; the areas of immediate contact of both ducts are much shorter in human than in chick embryos, as shown by the reconstructions published previously.<sup>1a</sup> However, another indication of very close relations of müllerian duct and mesenchyme stands out clearly in human embryos. The müllerian duct is for a long period not separated from the surrounding mesenchyme by a basal membrane. The same delicate fibers that are found throughout the mesenchyme are present at the boundary of the two tissues, and a few fibers appear to penetrate between the müllerian cells (fig. 3 *d*). This relation between an epithelium and the underlying mesenchyme is quite unusual and may well be an indication of an exchange of cells across the

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#### EXPLANATION OF FIGURE 2

Formation of mesenchyme from müllerian duct and tubal ridge in the chick embryo. Cross sections of the left müllerian duct and surrounding structures of an embryo of 6 days and 3 hours are shown in *a*, *b*, *c* and *d*; Gomori's silver impregnation. A cross section of the right müllerian duct and surrounding structures of a 6 day embryo is shown in *e*; Gomori's silver impregnation and acid fuchsin. Sections of the tubal ridge of an embryo of 7 days and 17 hours are presented in *f* and *g*; Gomori's silver impregnation. The tubal ridge of experimental embryo 499 is seen in *h*; Gomori's silver impregnation. See explanation in the text. *M* indicates the müllerian duct; *T*, the tubal ridge; *W*, the wolffian duct. Arrows point across the common basal membrane of the wolffian and müllerian ducts into the portions of the müllerian primordium being transformed into mesenchyme.



boundary. When the müllerian duct finally acquires a basal membrane, the surrounding mesenchyme forms at first a concentric layer similar to that shown in the chick embryo in figure 2 *d*.

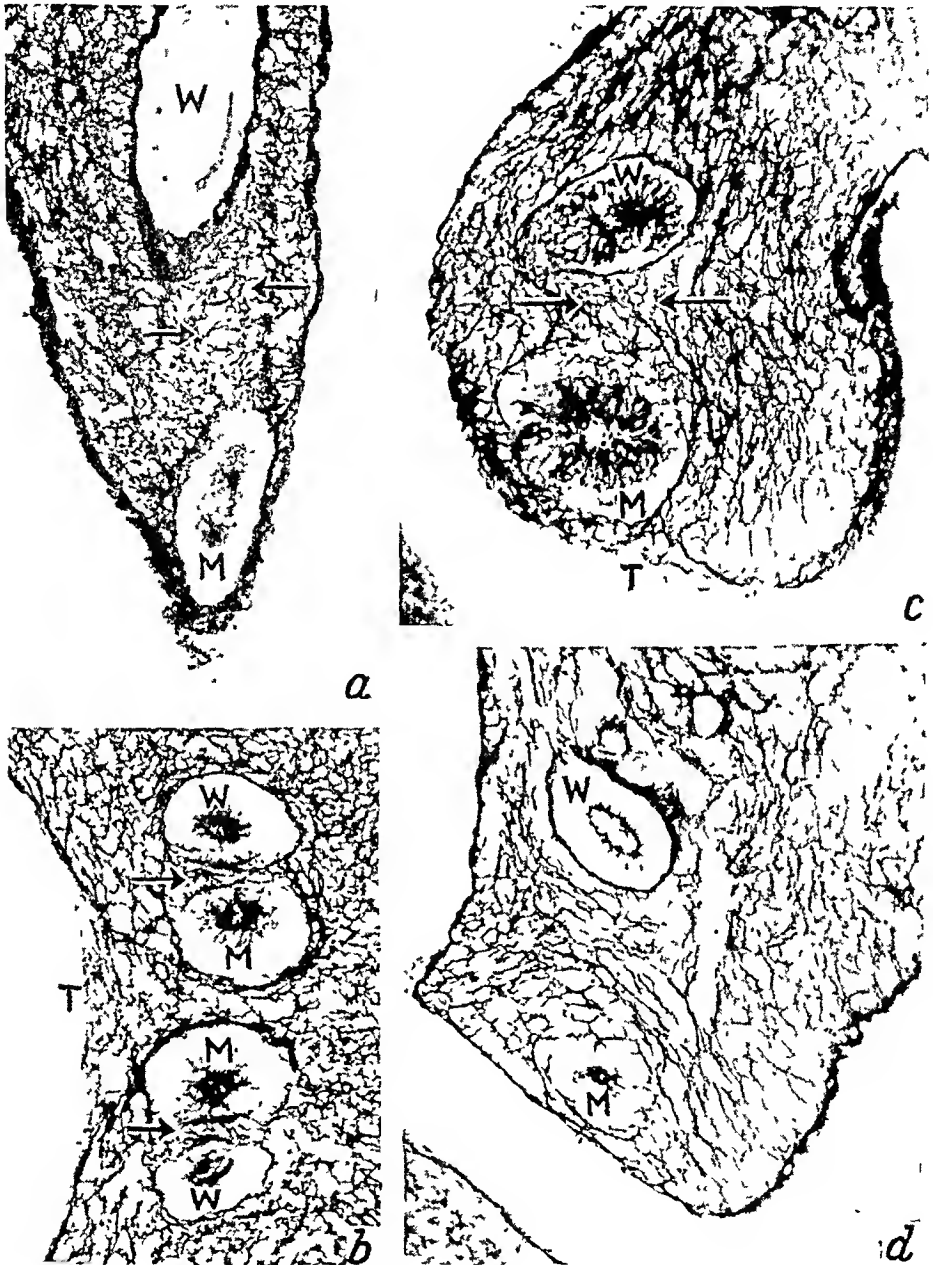


Fig. 3.—Formation of mesenchyme from müllerian duct and tubal ridge in the human embryo. Part *a* shows a cross section of a portion of the left urogenital ridge of a 21 mm. embryo. Parts *b* to *d* show sections from a 23 mm. embryo; in *b* one sees ducts of both sides in the pelvic region; in *c* and *d*, the left urogenital ridge; Gomori's silver impregnation. *M* indicates the müllerian duct; *T*, the area in which mesenchyme is in process of formation from the peritoneal epithelium (tubal ridge); *W*, the wolffian duct. Arrows point across the common basal membrane of the wolffian and müllerian ducts into the portion of the müllerian primordium being transformed into mesenchyme.

It has thus been demonstrated that part of the mesenchyme surrounding the müllerian duct in later stages originates directly from the epithelium of that duct. After the completion of this transformation the mesenchyme of müllerian origin cannot be distinguished from that previously present in this area.

*Mesenchyme Formation from the Peritoneal Epithelium.*—It was mentioned that the uniform condition of the early celomic wall is eventually replaced by one in which the superficial layer of cells is separated from the adjacent mesenchyme by its differentiation into a typical epithelium (fig. 1 *a* and *b*). However, a number of instances are on record to show that even in much later stages the celomic epithelium may contribute to the adjacent mesenchyme. The most impressive example is the transformation of the tubal ridge, and it is of particular interest for the present considerations because of the close proximity of the müllerian duct. The location of the tubal ridge and its relation to the müllerian duct were referred to earlier. It will now be shown that while this strip of peritoneal epithelium loses its tall appearance, the greater part of its cells are transformed into mesenchyme. These findings are also based on the material briefly described in the preceding section. Here, too, the more extensive changes in chick embryos will be described first.

The earliest indications of changes in the tubal ridge appear in chick embryos during the latter half of the sixth day. Silver impregnation reveals that argyrophil fibers appear in the basal portion of the tall epithelium, beginning in its cranial parts. As do most differentiations in this area, this process gradually proceeds in a caudal direction, and at the same time the fiber network increases in density in the cranial portions. Early stages of this process may be seen in figure 2 *c* and *d*, and still better in figure 2 *f*, showing the tubal ridge of a chick embryo of 7 days 17 hours. A network of argyrophil fibers spreads within the ridge distal to the basal membrane but never reaches the peripheral border of the epithelium. In a later stage, as shown in figure 2 *g* from a more cranial portion of the same embryo, the basal membrane of the tubal ridge disappears in a fiber network consisting in its proximal part (nearest the müllerian duct) of a small amount of mesenchyme, previously present in that location, and in its distal portion of transformed tissue of the tubal ridge. This transformed tissue soon assumes all the characteristics of the adjacent mesenchyme. Only a thin distal layer of the former tubal ridge remains epithelial and is eventually separated from the rest by a new basal membrane. It thus appears that a great part of the mesenchyme separating the peritoneal epithelium from the müllerian duct in later stages is derived from the epithelial tubal

ridge. The just described transformation is finished in the chick embryo during the ninth day of incubation.

In the human embryo the tubal ridge never develops as tall as in the chick, and consequently the changes are not so pronounced. However, there is evidence of transformation of epithelial cells into mesenchyme in the corresponding areas of the human embryo, too. In figure 3 *b* and *c*, for instance, there is shown a condition of the peritoneal covering of the müllerian duct which is highly indicative of fiber formation in the original epithelium. Similar observations were made in various mammalian embryos, revealing varying degrees of transformation of the tubal ridge into mesenchyme.

A series of experiments was briefly referred to on an earlier page, in which the urogenital ridge contained no müllerian duct as a consequence of interruption of the wolffian duct in an early stage.<sup>5</sup> In order to follow the changes in the tubal ridge in these specimens, sections were restained to show argyrophil fibers. Figure 2 *h* shows the wolffian duct and tubal ridge on the left side of embryo 499. The changes in the tubal ridge correspond exactly to those just described, and the old basal membrane is just about to disappear in the network of newly formed fibers. The müllerian duct is absent as a result of the experiment. This shows that the transformation of part of the tubal ridge into mesenchyme does not depend on the presence of that duct as an organizer. Only in those cases in which the entire urogenital ridge (with the exception of the gonad) is absent as a consequence of destruction of the wolffian duct is there no tubal ridge and no mesenchyme formation from the peritoneal epithelium.

Thus the mesenchyme formed in early embryonic stages from the celomic lining (fig. 1 *a*) and later from the müllerian epithelium (fig. 1 *d*, *f*, *g* and *h*) is supplemented from a third source, namely, the tubal ridge (fig. 1 *d*, *e* and *f*). It was pointed out earlier in this report that the mesenchyme of the entire celomic wall and its derivatives is in its development and potencies unusually closely related to the epithelial components of these structures. This is due to the early uniform condition of the celomic wall and some of the organ primordia forming in it. However, in the vicinity of the müllerian duct these relations of mesenchyme and epithelium are still closer because much of that mesenchyme develops in late stages from the well differentiated epithelia of the müllerian duct and the nearby peritoneum.

In terms of the morphology of adults these facts mean that the non-epithelial layers of the uterovaginal canal, including the lamina propria, the muscularis and the subserous tissue, originate at least partly from cells which had been parts of the inner or the outer epithelial lining.

How much of the potencies of these epithelia is retained in the other cells of the uterovaginal canal is not known. In view of the embryologic facts described in the preceding pages, the existence of such potencies in the nonepithelial cells of this tract must be considered possible if not probable.

#### CONCLUSIONS REGARDING REGENERATIVE AND PATHOLOGIC GROWTH IN THE UTEROVAGINAL CANAL

New possibilities for the understanding of growth in the uterovaginal canal arise from the fact that latent potencies may be widely distributed in its tissues and embryologically related areas, irrespective of epithelial or nonepithelial tissue structure. The possibility that uterine epithelium may originate from mesenchyme was extensively discussed by Heim<sup>6</sup> in connection with the problem of endometriosis. However, the considerations of this author are embryologically ill founded, and the formation of mesenchyme from the müllerian duct itself was unknown at the time of his publication. With the facts now available, a conception of the possible role of mesenchyme in the formation of uterine epithelium can be developed on a sound embryologic basis.

*Physiologic Regeneration of the Endometrium.*—No part of the body undergoes regeneration as frequently and extensively under normal conditions as the endometrium. It is almost generally assumed that during this regeneration all cells develop from their equals; that is, epithelial cells from the lining of the glands, and connective tissue by multiplication of the surviving stroma. Papanicolaou,<sup>7</sup> after studying this process in the guinea pig, arrived at a different conclusion. According to him, on the surface of the endometrium, as well as in many glands whose epithelium had been cast off, the superficial stroma cells differentiate into a new epithelium. This is far from being conclusively proved, but it has not even been discussed extensively, owing, probably, to a deep-rooted aversion to the assumption that epithelium may be formed from connective tissue in the adult organism. The present work cannot solve this problem, but it lays the embryologic foundation for further investigation. The fact that the stroma of the endometrium contains cells which are derived from the müllerian duct makes it appear quite possible that these cells have the potency to form uterine epithelium, just as did the duct from which they originated. Future investigators of postmenstrual and puerperal changes in the endometrium will have to give this possibility more consideration than has been done up to the present time.

6. Heim, K.: Arch. f. Gynäk. **152**:269, 1933.

7. Papanicolaou, G. W.: Am. J. Obst. & Gynec. **25**:30, 1933.

*Endometriosis.*—In the vast literature concerned with the origin of endometriosis many attempts were made to justify the assumption that various tissues may be transformed into uterine mucosa. Thus the adherers to the theory of local origin of ectopic endometriosis (in contrast to the transportation of germs from the endometrium) included in their consideration first the entire celomic epithelium, then the mesenchyme of certain parts of the celomic wall (Heim<sup>6</sup>) and finally the entire mesenchyme of the body (Biebl<sup>8</sup>). This last expansion was precipitated by the discovery of endometriosis in the arm and the leg. In consideration of the present state of embryologic knowledge, a critical review of the subject leads to the following conclusions (Gruenwald<sup>4</sup>): It is true that the celomic epithelium, being the mother tissue of the müllerian ducts, must be regarded as a possible source of endometriosis in the first place. The mesenchyme of the celomic wall, whose intimate developmental relations to the epithelium have been referred to several times in the present report, should be taken into account along with the celomic epithelium. There is no reason to exclude the cranial parts of the celomic wall, as did Heim.<sup>6</sup> One portion of the mesenchyme deserves special mention; this is the one developing directly from the epithelium of the müllerian duct. Its possible role in the regeneration of the endometrium was emphasized in the foregoing section, and it is of similarly great importance in the explanation of endometriosis. There is little reason to go as far as did Biebl<sup>8</sup> and include more than the mesenchyme of the celomic wall in these considerations, since the occurrence of endometriosis in the extremities can easily be accounted for on the basis of local origin from tissue of the celomic wall (Gruenwald<sup>4</sup>). It should be clear that this discussion is concerned only with the embryologic possibilities of explaining endometriosis by local differentiation; it cannot directly disprove the validity of other theories.

*Tumors of the Internal Genital Organs.*—The present facts and similar findings regarding the development<sup>1b</sup> of the adrenals and the gonads suggest that the unusually large variety of tumors related to these organs and the difficulties in their interpretation may well be due to the unusually wide range of structural variability in the tissues of celomic derivatives. In accordance with the distinctly and permanently epithelial or nonepithelial structure of most other tissues, tumors are customarily classified as epithelial or nonepithelial as well. Facing cells with labile structure as one does in the derivatives of the celomic wall, one must expect to find similar structural lability in the tumors arising from these cells. At the present time, only the suggestion can be made that tumors which cannot be definitely classified as either epithelial or nonepithelial may develop from celomic derivatives, as has

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8. Biebl, M.: Zentralbl. f. Chir. 65:1026, 1938.

already been indicated by Klemperer and Rabin<sup>9</sup> with regard to neoplasms of the pleura. Future investigations carried out with this possibility in mind will have to show whether or not the usual classification can be kept up in tumors of the structures in question, including the uterovaginal canal, gonads and adrenal cortex. If any difficulties of classification should appear, or the possibility suggest itself that epithelial tumors may have arisen from the nonepithelial tissues or vice versa, one should keep in mind that this may be a consequence of the peculiar development of the tissues concerned, and abstain from forcing such observations into the customary rigid system.

#### SUMMARY

A review of earlier and new embryologic findings is made in order to demonstrate the unusually intimate developmental relations of epithelial and mesenchymal constituents in the derivatives of the embryonic celomic wall. In these structures, which include the müllerian ducts, the gonads and the adrenal cortices, in addition to the serous membranes themselves, transitions between epithelial and nonepithelial components may be found during various stages of development up to the adult condition.

The formation of mesenchyme from the epithelium of the müllerian duct is described in detail. It occurs near the caudal end of the duct while this is growing toward the urogenital sinus. Similar formation of mesenchyme also takes place at the expense of the nearby peritoneal epithelium, the so-called tubal ridge. Consequently, the nonepithelial tissues of the uterovaginal canal arise not only from the mesenchyme originally present in that area but also in part from the epithelia of the inner and outer linings of the canal. Thus a strong possibility exists that these nonepithelial cells may possess the developmental potencies of their epithelial coverings. These facts make reports of postmenstrual regeneration of uterine epithelium from the stroma appear less improbable than is generally assumed. Furthermore, the possibility must be taken into account that a clearcut distinction of epithelial and nonepithelial structure may not always be possible in tumors of derivatives of the celomic wall. Endometriosis may develop not only from the celomic epithelium but also from the adjacent mesenchyme which is closely related to this tissue in its development. Thus endometriosis can be accounted for in all known locations by local differentiation of tissues derived from the celomic wall.

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9. Klemperer, P., and Rabin, C. B.: *Arch. Path.* **11**:385, 1931.

# LATENT PRIMARY CARCINOMA

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Standard textbooks of pathology yield little information concerning latency in cancer. Until Willis<sup>1</sup> published his admirable treatise, knowledge of latent carcinoma existed only in the form of isolated case reports. Willis presented a detailed and complete bibliography with an orderly segregation of the reported cases and his own cases according to primary sites. In the present paper an attempt will be made to record a group of cases of latent primary cancer observed during a five year period in a general hospital for patients with acute diseases, to define and establish limitations for the concept of latency and finally to evaluate, if possible, the factors which tend to make any tumor a latent one.

The term "latency" has never been adequately defined. Until the exact limitations of the term are agreed on, the results of studies of the incidence of latent cancer cannot become comparable. Encompassed in this term are variable and conflicting clinical and pathologic concepts. Clinically, it includes thoughts that range over a wide sphere, such as: inaccessibility to clinical examination or to biopsy; general difficulty in establishing a diagnosis, as in cases in which the tumor simulates other disease entities; gross error in diagnosis; presence of general symptoms of cachexia initiating a suspicion of cancer, with or without localizing symptoms; a case of cancer in which the picture is confused by an additional unrelated disease; a tumor which plays no part in or is quite secondary to the causation of death; a cancer which tends to grow rapidly or to metastasize early. The term "latent" is used loosely by clinicians to include one or more of the foregoing categories.

A wide latitude is also observed in the interpretation of latency from the point of view of pathology. The term is used to include: an asymptomatic cancer with or without metastasis which is discovered incidentally either in the gross or the microscopic study of the viscera

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1. Willis, R. A.: *The Spread of Tumours in the Human Body*, London, J. & A. Churchill, 1934, chap. 12, pp. 179-202.

at autopsy; a cancer whose initial manifestation is due to some dramatic local complication, such as hemorrhage or obstruction; a primary cancer which has invaded neighboring organs, with symptoms due to involvement of the latter; a noncancerous tumor becoming cancerous; a cancer whose growth is extremely slow or stationary; a cancer whose primary site cannot be demonstrated clinically; a cancer whose metastasis is more anaplastic and outstrips the primary growth, and finally, *a cancer whose metastasis causes the initial symptomatic picture.*

Included in the last category are most of the tumors collected and discussed by Willis. Our material, as the title of this article implies, has been selected on the same basis, so well paraphrased by Willis in chapter 12 of his book: "a highly important group of symptomatically quiescent primary tumors in which the attention of the clinician is focused on precocious metastases." Twenty-five such cases of latent primary cancer were encountered in a series of 2,514 autopsies, from November 1935 to December 1940, inclusive, at the Queens General Hospital.

In every case the initial symptoms complained of by the patient were referable either directly or indirectly to the metastasis. In some instances the patient gave a history of some symptoms referable to the primary site before entry into the hospital, but in all such instances these symptoms were preceded, by a distinct interval, by complaints due to the precocious metastasis. A correct diagnosis of the primary site was made in 2 cases on the basis of a biopsy of material obtained from the metastatic focus. All the other clinical and pathologic categories of latency listed are eliminated from this discussion. Only cases studied at autopsy are included, and all diagnoses were confirmed histologically.

These 25 cases with the presenting clinical complaints, the symptomatic metastatic sites and the corresponding asymptomatic primary sites are listed in table 1. There is a definite need for this type of information obtained in a uniform manner and permitting summation and statistical comparison and analysis.

#### REPORT OF CASES

CASE 1.—A 56 year old white woman entered the hospital because of headache of one week's duration and vomiting for two days. The day before admission she complained of a severe dizzy spell with loss of vision lasting several minutes. The pupils were irregular and fixed to light but reacted to accommodation. There was central paresis of the right side of the face. The abdominal reflex on the left was absent. A spinal tap revealed markedly increased pressure of the spinal fluid. The patient suddenly became comatose, the pulse rate dropped to 36 per minute and the respiration was markedly slowed. She quickly regained her normal state and was not aware of the episode. A marked increase in papilledema was noted. A ventriculogram showed no dilatation or distortion of the ventricular system. The patient showed progressive disorientation and increasing stupor. Another ventriculogram one month after admission



TABLE 1.—*Summary of Twenty-Five Cases of Latent Carcinoma*

Case	Complaint	Clinical Diagnosis	Secondary Site	Primary Site
1	Headache and vomiting.....	Cerebellar tumor .....	Meninges with occlusion of foramina of Magendie and Luschka and hydrocephalus	Stomach—diffuse colloid cancer
2	Headache, dizziness, ataxia.....	Chronic sinusitis; bilateral bronchiectasis; abscess of posterior fossa of brain	Cerebellum and cerebrum.....	Left upper lobe bronchus—adenocarcinoma
3	Girdle abdominal and back pain and urinary incontinence	Tumor of spinal cord; possible cordary occlusion	Thoracic extradural tissues with compression of spinal cord	Prostate—adenocarcinoma
4	Right hemiparesis discounted because of the presence of advanced multiple sclerosis	Advanced multiple sclerosis; bronchopneumonia	Left frontal lobe.....	Fundus of uterus—anaplastic adenocarcinoma
5	Headache, vomiting, coma.....	Cerebral thrombosis due to arteriosclerosis	Brain.....	Bronchus—anaplastic squamous carcinoma
6	Fracture of right femur.....	Hypernephroma of right kidney; metastasis to right femur; pathologic fracture of the right femur	Right femur.....	Right kidney—hypernephroma
7	Pain in left knee.....	Metastatic carcinoma of head of fibula, probably from stomach; carcinomatosis	Head of left fibula.....	Pancreas—papillary cyst—adenocarcinoma of head
8	Pathologic fracture of left femur....	Gastrointestinal cancer with metastasis to left femur; ununited fracture of left knee	Left femur.....	Stomach—anaplastic adenocarcinoma of fundus
9	Pain in sternum and occipital headache	Metastatic carcinoma of sternum and frontal bones, primary site undetermined, probably bronchiogenic; cachexia	Sternum and brain.....	Right main bronchus—adenocarcinoma
10	Abdominal pain due to intestinal obstruction	Intestinal obstruction due to carcinoma of unknown primary site	Peritoneum and ileum with obstruction	Tail and body of pancreas—adenocarcinoma
11	Ascites.....	Thrombophlebitis of left leg; embolism of pulmonary artery; ascites of unknown cause	Peritoneum.....	Undetermined; erroneously considered tuberculosis at time of gross autopsy
12	Epigastric pain and psychosis.....	Mediastinal mass of questionable cause; general and cerebral arteriosclerosis with psychosis	Peritoneum and brain.....	Bronchus to upper lobe of right lung—adenocarcinoma

13	Ascites.....	Cirrhosis of the liver with ascites; coronary thrombosis	Peritoneum.....	Pancreas—adenocarcinoma
14	Left pleural effusion.....	Probable bronchiogenic adenocarcinoma; metastasis to the pleura; spontaneous left hydropneumothorax	Left pleura.....	Ovaries—papillary pseudomucinous cyst—adenocarcinoma
15	Dyspnea and thoracic pain.....	Bronchiogenic carcinoma with pleural effusion; pneumococcal sepsis	Pleura.....	Prostate—adenocarcinoma
16	Cough, pain in chest.....	Bronchiogenic carcinoma; phlegmon of right wall of chest	Pleura and lung.....	Male breast—duct carcinoma
17	Mass in left inguinal region, one year?	Carcinoma of stomach with metastasis; general peritonitis following perforation of carcinoma; left inguinal hernia (based on exploratory laparotomy)	Inguinal lymph nodes.....	Stomach—adenocarcinoma of pylorus
18	Cough and hemoptysis.....	General lymphosarcoma, possibly Hodgkin's disease	Mediastinum.....	Body of pancreas—medullary carcinoma
19	Enlargement of neck.....	Carcinomatosis, primary site undetermined	Cervical lymph nodes.....	Undetermined clinically and at autopsy
20	Epigastric pain.....	Bronchiogenic carcinoma with metastasis to liver	Liver.....	Bronchus to middle lobe of right lung—reserve cell carcinoma
21	Epigastric distress.....	Gastrointestinal cancer, exact site undetermined, with metastases to liver and lung	Massive involvement of liver.....	Bronchus to lower lobe of right lung—anaplastic squamous cell carcinoma
22	Hemoptysis.....	Possible subacute yellow atrophy; pulmonary or gastric hemorrhage	Lung.....	Left testicle—choriocarcinoma
23	General peritonitis secondary to perforated metastatic carcinoma of ileum	Malignant tumor of ileum.....	Ileum.....	Bronchus—anaplastic squamous cell carcinoma
24	Edema of right lower extremity.....	Neoplasm of unknown primary site; obstruction of venous and lymphatic return from right lower extremity by neoplasm	Ovaries with stasis of iliac veins and thrombosis (Krukenberg)	Stomach—cellular fibrocarcinoma
25	Epigastric pain.....	Osteogenic sarcoma of left tibia with metastases to liver and lung	Right adrenal.....	Bronchus to lower lobe of left lung—oat cell carcinoma

demonstrated moderate dilatation of both lateral ventricles with no deviation from the midline. Suboccipital craniotomy was begun for tumor of the posterior fossa, in the course of which the patient suddenly went into vascular collapse.

At autopsy colloid carcinoma, diffuse in type, was found in the pyloric antrum. Gross examination of the brain disclosed no abnormality other than dilatation of the ventricles. Microscopic examination, however, revealed extensive carcinosis of the meninges with prominence of signet ring cells (fig. 1).

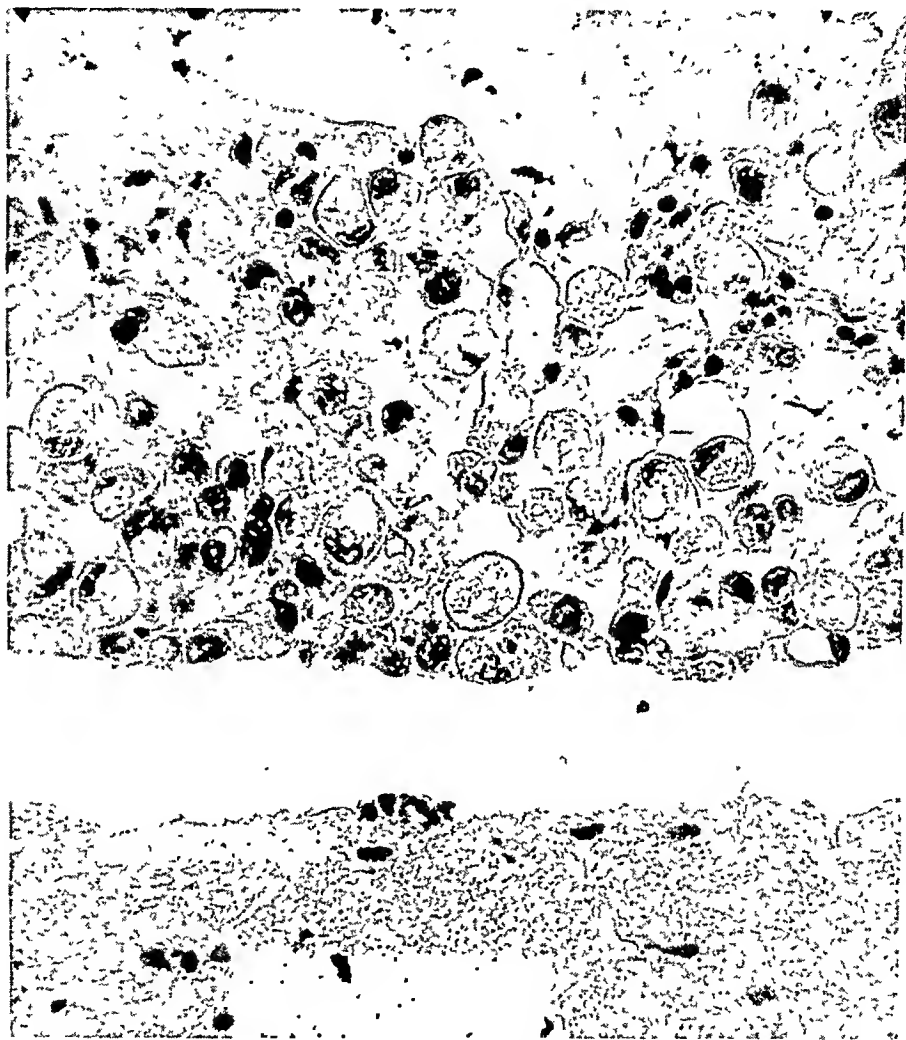


Fig. 1 (case 1).—Section of brain and leptomeninges showing diffuse carcinosis of the meninges with signet ring cells recognizable;  $\times 200$ .

CASE 2.—A 56 year old white man entered the hospital with a three week history of headaches, nausea, anorexia and loss of weight. In addition, he had noted staggering gait, falling most often to the left and difficulty in talking. Occasional dizziness and progressive loss of memory developed. Chronic sinusitis with a purulent discharge in the nasal passage was noted. The deep reflexes were overactive, more marked on the left. The left abdominal reflex was absent, and there were present Babinski's reflex, nystagmus, past pointing with the left

hand, a defective heel to knee test on the left, adiodokokinesis on the left and weakness of grasp with the left hand. Sphenoethmoidectomy was performed, with no relief of symptoms. A roentgenogram of the chest was then interpreted as evidencing possible atelectasis on the basis of a primary bronchiogenic tumor. Bronchoscopic inspection revealed no sign of endobronchial neoplasm. There was moderate bronchiectasis. The patient went downhill rapidly and died nine days after admission.

Autopsy revealed a small tumor of one of the bronchi to the peripheral part of the upper lobe of the left lung. The entire mass measured 2 by 1 cm. and was located immediately beneath a depressed, puckered, scarred area in the visceral pleura. A solitary small mediastinal lymph node showed a miniature area of metastasis. The left cerebellar hemisphere presented a large mass, measuring 3 by 2 by 1 cm., which protruded above the level of the pia-arachnoid. There was a large cystic area of metastasis in the occipital lobe, as well as many smaller scattered areas of tumor tissue throughout the remainder of the cerebrum.

CASE 3.—A 61 year old white man was admitted with a history of burning pain over the sternum, radiating to both the arms and the back, and a girdle-like pain in the lower part of the abdomen of six months' duration, with almost simultaneous onset of urinary incontinence and dysuria. For the past two months he had been constipated. Eleven days before admission he noted sudden onset of weakness in both legs, followed in rapid sequence by complete paralysis and incontinence of feces. The deep reflexes in both lower extremities and vibratory sense on the left were absent, and a sensory level was found at the third thoracic segment. Rectal examination was not done. Three days after admission the patient suddenly gasped and ceased breathing.

Autopsy revealed a flat firm tumor mass in the extradural tissues in the region of the third thoracic vertebra. This mass completely encircled the spinal cord. Section revealed softening and hemorrhage within the cord substance. A large firm mass was found completely replacing the prostate, with extensive regional involvement.

CASE 4.—The patient was a 28 year old white woman with multiple sclerosis of at least three years' duration. During these three years there had been remissions and exacerbations. During the ten days before admission to the hospital she took a sudden turn for the worse, was unable to walk, became incontinent of urine and feces and presented palsy of the right side of the face. There were marked nystagmus, bilateral temporal pallor of the optic disks, a monotonous tone to the speech, central palsy of the right side of the face and marked weakness of the right upper and lower extremities. The deep reflexes were overactive, the right more than the left, with bilateral Babinski reflexes. Position and vibratory sense were impaired, especially on the left. A decubital ulcer of the heel developed, and the patient rapidly grew worse and died in seventeen days after admission.

Autopsy revealed evidence of disseminated sclerosis with involvement of both the brain and the spinal cord. In addition, a large isolated tumor mass, measuring 4.5 by 5.5 cm., was seen in the left frontal lobe and in the region of the basal ganglions. The uterus showed a small reddish gray fleshy mass projecting into the endometrial cavity from the posterior surface of the endometrium at the fundus. No other metastases from this primary site were found.<sup>2</sup>

2. Mitchell, N., and Angrist, A.: *Am. J. Clin. Path.* **12**:232, 1942.

CASE 5.—A 60 year old white woman was admitted in coma, with a history of sudden collapse while in bed. During the six months before admission there had been definite change in personality associated with severe intermittent headaches, nausea and vomiting. Hemiparesis and Babinski's reflex were found on the left side. The patient regained consciousness on the day after admission. Five days later, bilateral papilledema was noted. Fifteen days after admission, the patient again lapsed into coma, and decompression was carried out for a probable tumor of the brain. She died two weeks after operation.

Autopsy revealed a cystic cavity in the right frontal lobe, measuring 6 cm. in diameter, with central softening. Dissection of the right lung revealed a peripherally placed tumor mass in the upper lobe with a softened center and with carcinomatous tissue lining the wall of the cavity. Suppuration was noted in both the pulmonary tumor and the metastatic tumor of the brain.

CASE 6.—A 59 year old white woman was transferred from another hospital for radiation therapy. She had suffered a fracture of the right femur in a fall one month before. An osteolytic area suggestive of metastatic carcinoma was seen in the roentgenogram. A punch biopsy of the involved femur revealed metastatic tumor which suggested hypernephroma as the primary site. No definite history of hematuria was elicited. Intense high voltage irradiation of the right femur was begun. Repeated urinalysis, prompted by the biopsy report, showed occasional hematuria. Retrograde urography revealed definite distortion of the pattern of the right renal pelvis, and a diagnosis of hypernephroma of the right kidney was made. The patient complained of an occasional pain at the site of fracture. She was discharged four months after admission. Symptoms suggesting ascending urinary infection following complete transection of the spinal cord by metastatic carcinoma appeared, and death occurred two months later.

At autopsy, the right kidney showed one large and several small satellite masses; the largest, measuring 5 cm. in diameter, occupied the midzone of the kidney. These masses were soft, opaque and yellow and were clearly demarcated from the regional parenchyma. The lower third of the right femur presented a large mass of metastatic tumor tissue, which invaded the medullary cavity of the bone. Extensive metastases were also found in the lower thoracic vertebrae with direct extension through the vertebral bodies to the extradural tissue and compression of the spinal cord.

CASE 7.—A 67 year old white man struck his left knee against a rock seven weeks prior to admission, and subsequently there were recurrent episodes of progressively increasing pain in the region of the left knee. Anorexia, nausea and vomiting appeared in the few weeks prior to admission. A large mass in the right upper quadrant of the abdomen was apparently liver. A large mass was felt in the region of the head of the left fibula. Roentgen examination of the left leg revealed a large area of rarefaction in the head of the left fibula and a large soft tissue mass. A barium sulfate enema revealed displacement downward of the transverse colon by the large abdominal mass. The patient declined rapidly and died within a week.

At autopsy there was a large mass, measuring approximately 10 cm., in the head of the pancreas, with regional extension to the walls of the stomach and duodenum, and there were bulky metastases in the liver and in the head of the left fibula.

CASE 8.—A 61 year old white man was first observed because of soreness and pain in the left hip and the sensation of a sudden "snap" in the left lower extremity. Roentgen examination revealed a fairly well demarcated osteolytic area in the upper part of the neck of the femur with an ununited fracture. The man improved somewhat, but returned, seven months later, with a huge mass in the left thigh, extending from the inguinal ligament to a point just above the knee. In addition, a large firm mass was felt occupying the entire left half of the upper part of the abdomen. His condition grew rapidly worse, a spiking fever developed, and death occurred four weeks after the last admission.

Autopsy revealed a large ulcerating carcinoma of the fundus of the stomach, with regional extension to the spleen and a gastrosplenic fistula, and a huge metastatic tumor in the left femur and regional tissue.

CASE 9.—A 50 year old white woman was admitted with complaints of pain in the sternum and occipital headache of three months' duration. There had also developed a tender lump on the frontal bone and pain in the left shoulder and axilla. Dulness over the lower lobe of the left lung and diminished breath sounds over the upper lobe of the right lung were noted. Biopsy of the sternal mass revealed metastatic adenocarcinoma. A probable diagnosis of bronchiogenic carcinoma was made, but the patient was too weak to be examined bronchoscopically. She died, after a gradual decline, two months later.

Autopsy revealed carcinoma of the right main bronchus with extensive bony and visceral parenchymal metastases.

CASE 10.—A 58 year old white man was admitted with colicky abdominal pain of five weeks' duration, associated with constipation, and obstipation for two days. The abdomen was diffusely enlarged, and there was an indefinite mass in the epigastrium. Conservative therapy with Wangensteen suction and colonic irrigations were ineffectual. Exploratory laparotomy revealed diffuse peritoneal carcinosis. Biopsy of the omentum showed metastatic adenocarcinoma, suggesting pancreatic origin. The patient died three days after operation.

Autopsy revealed a large firm mass in the tail and body of the pancreas with extensive peritoneal implantation and extension to many segments of the ileum. The involvement of the small intestine was marked by stenosis and by penetration to the mucosa, with occasional ulceration.

CASE 11.—The patient was a 38 year old white man. Three weeks before admission he noted sudden onset of swelling of the abdomen. This was progressive. Five days before admission he noted pain in the left leg, accompanied by swelling. The abdomen was markedly distended, with definite shifting dullness. Tenderness and swelling of the entire left lower extremity were present. Shortly after admission the patient became markedly dyspneic and apprehensive, went into shock and died within an hour.

Autopsy revealed multiple small whitish nodules covering the entire extent of the peritoneum. No tumor mass was obvious in the abdominal or the thoracic viscera. On the gross appearance a diagnosis of tuberculous peritonitis was made. Microscopically, the whitish nodules were composed of adenocarcinomatous tissue suggesting either gastric or pancreatic origin. In addition, left femoral thrombophlebitis and massive pulmonary embolism were found.

CASE 12.—A 59 year old white man was admitted complaining of epigastric pain and loss of weight. The stools had been alternately tarry and clay colored.

A resistance was felt in the right upper quadrant of the abdomen. A large mass was present in the lower portion of the neck. Enlargement of the cervical and supraclavicular lymph nodes was noted. A roentgenogram of the esophagus revealed obstruction at the fourth dorsal vertebra by an extrinsic mediastinal mass. The patient became disorderly and disoriented, requiring large doses of sedative. Bronchoscopic examination revealed no evidence of an intrinsic bronchial neoplasm.

Autopsy revealed carcinoma of the bronchus to the upper lobe of the right lung and massive involvement of mediastinal lymph nodes with obstruction of the superior vena cava and local infiltration into the pericardium. The esophagus was displaced posteriorly.

CASE 13.—A 60 year old white man had swelling of the abdomen of two weeks' duration. There was a small liver, and abdominal paracentesis yielded 2,600 cc. of straw-colored fluid. Four days after admission the patient suddenly collapsed and died within a few minutes.

Autopsy disclosed marked interstitial pancreatitis and adenocarcinoma of the pancreas with massive carcinomatosis of the peritoneum and ascites. Grossly the pancreas showed hyperlobulation with no definite tumor nodules, but microscopically the cancerous features were obvious.

CASE 14.—A 58 year old white woman was first observed in 1936 because of increasing severe dyspnea of several months' duration. At that time pleural effusion was found on the left, which was repeatedly tapped, with improvement. The pleural fluid contained grouped cells of adenocarcinoma. During the subsequent four years the patient underwent thoracentesis innumerable times and received large doses of high voltage roentgen radiation. During this interval extensive clinical and laboratory investigations yielded no definite diagnosis of the primary site. The patient was admitted to the hospital for the last time in 1940 and died after a relentless downhill course, marked terminally by sudden onset of pain in the left side of the chest and severe dyspnea, accompanied by hyperresonance and diminished breath sounds on the left, interpreted as spontaneous pneumothorax.

Autopsy revealed hydropneumothorax on the left. The pleura on the left side was markedly thickened, and the left lung was markedly shrunk and collapsed. No primary tumor was found after careful dissection of the bronchi. The ovaries were moderately enlarged and revealed primary papilliferous, opaque, grayish white tissue completely replacing the ovarian stroma. The pleura was markedly fibrotic and hyalinized with metastatic carcinoma discernible in a few places.

CASE 15.—A 64 year old white man was admitted with a history of dyspnea and pain in the lower part of the right side of the chest of six months' duration. Paracentesis for pleural effusion on the right had been done repeatedly in the six month interval. Repeated examinations of sputum, bronchography with iodized poppy seed oil and bronchoscopy yielded no definite information as to the cause of the pleural effusion. No other thoracic symptoms and no urinary complaints were noted. The course was steadily downhill with development of Pneumococcus type 33 sepsis terminally.

Autopsy showed marked thickening of the pleura on the right with limited extension to the periphery of the lower lobe of the right lung. After careful dissection of the bronchi, no definite primary site was noted. The prostate grossly

was of normal size, was somewhat indurated and presented many greenish black calculi. Microscopically there was diffuse adenocarcinoma of the prostate with marked perineural lymphatic invasion. The pleura as well as the lung showed metastatic adenocarcinoma. Empyema of the right pleural cavity was found, yielding *Pneumococcus* type 33 on culture.

CASE 16.—A 45 year old Negro man complained of cough of eight months' duration, associated with pain in the right side of the chest, malaise and night sweats. Diminution of breath sounds over the entire right side of the chest was noted, and coarse rales were heard over the bases of the lungs. The sputum was negative for acid-fast bacilli. A pleural effusion on the right was aspirated, with considerable relief. Two weeks after admission there were induration and edema of the right side of the chest beneath the region of the right nipple, and the edema and redness increased. A definite mass could not be outlined. Bronchoscopic examination was withheld because of the rapid onset of marked weakness and cachexia.

Autopsy revealed adenocarcinoma infiltrating the right breast and the regional pectoral muscles, metastasis to the axillary lymph nodes and widespread pleural carcinomatosis of the right lung. No intrabronchial tumor tissue was identified. A large abscess cavity was noted in the upper lobe of the right lung, and empyema was present in the right pleural cavity.

CASE 17.—A 68 year old white woman entered the hospital with the complaint that a mass had been present in the left inguinal region for the past four years. This mass had been reducible by means of a truss except in the last year. About seven weeks before admission to the hospital she complained of intermittent pain in the region of the mass and vague pain in the upper part of the abdomen. The latter was associated with anorexia, weakness and constipation. She had had dark stools recently. A large liver, a mass in the epigastrium and left indirect inguinal hernia were noted. Six days after admission she had sudden onset of general abdominal pain, associated with tenderness and rigidity in the upper abdominal region. Laparotomy revealed a large lesion of the stomach with perforation, diagnosed as carcinoma.

At autopsy a large mass, 10 cm. in diameter, was observed in the pyloric region, with perforation and general peritonitis. Adherent omentum was seen in the region of the gastroduodenum. It was established that the mass in the inguinal region was a metastasis to the left inguinal nodes.

CASE 18.—A 38 year old white man was admitted with a history of cough since September 1938 and of blood streaking of the sputum for two weeks. In addition, he had progressive loss of weight, night sweats and dyspnea. Increased retrosternal dullness was noted. A roentgenogram of the chest showed marked widening of the mediastinal and pericardial shadows. The liver and the spleen were moderately enlarged. Firm axillary lymph nodes were palpated. The patient pursued a gradual downhill course and died eight weeks after admission.

Autopsy revealed extensive tumor infiltration of all the mediastinal structures with involvement of the hilus of each lung and the pericardium. A huge retroperitoneal mass showed complete destruction of the body and tail of the pancreas and replacement by opaque grayish tumor tissue. The head of the pancreas showed the usual markings.

CASE 19.—A 47 year old white man was admitted because of marked enlargement of the right side of the neck for three months and recent pain in the right



flank, treated as a renal ailment. Dysphagia and dyspnea had been noted only during the past three to four weeks, associated with a loss of 25 pounds (11 Kg). The neck showed large masses, fixed, confluent, rapidly growing, which first appeared in the upper nodes and then in the lower. The diagnosis of Hodgkin's disease or lymphosarcoma was considered until biopsy, concurred in by several pathologists, established the diagnosis as metastatic anaplastic carcinoma. Bronchoscopic examination and careful study of the nasopharynx never revealed the primary tumor.

At autopsy the primary site was not established, as permission had been given for a limited examination only. The head, nasopharynx and neck were not explored. Extensive anaplastic squamous carcinoma metastatic to the adrenals and general metastasis to the cervical, mediastinal, mesenteric and retroperitoneal nodes were found.

CASE 20.—A 62 year old white man complained of abdominal discomfort and pain of three weeks' duration associated with constipation and light yellow stools. A loss of 35 pounds (16 Kg.) during the past four months was noted, and abdominal enlargement was seen within the last few weeks. The pertinent findings were an enlarged liver, extending 5 fingerbreadths below the costal margin, a sense of a large mass in the epigastrium and diminished breath sounds over the apex of the right lung anteriorly with dulness over the right half of the chest. A roentgenogram of the chest revealed a large triangular area of consolidation extending outward from the region of the right hilus, suggesting cancer of the bronchus to the middle lobe of the right lung. A small amount of bloody fluid was aspirated from the right pleural cavity. The patient grew rapidly worse and died thirteen days after admission.

At autopsy an ulcerated cancer of the proximal portion of the bronchus to the middle lobe of the right lung was seen with infiltration of the surrounding parenchyma over an area measuring 3 cm. in diameter. The regional lymph nodes were enlarged and contained metastatic tumor tissue. The liver was markedly enlarged, weighing 3,410 Gm., and contained innumerable small and large metastatic carcinomatous nodules.

CASE 21.—A 50 year old white man complained of epigastric distress of three months' duration, accompanied by loss of weight, anorexia and light yellow stools. There was a large tense mass in the epigastrium and right upper quadrant of the abdomen, with diminished breath sounds and dulness over the entire right lung. A roentgenogram of the chest revealed a dense shadow covering the entire right lung field. Aspiration of the right pleural cavity yielded 2,000 cc. of frankly purulent material. A gastrointestinal roentgenogram series showed no evidence of intrinsic neoplasm of the stomach. Despite thoracotomy and removal of large amounts of purulent material from the right pleural cavity, the patient went gradually downhill and died two months after entering hospital.

At autopsy an ulcerating tumor was found in the bronchus to the lower lobe of the right lung, with extension to the regional pulmonary parenchyma and marked infiltration of the entire pleura, and a well developed empyema cavity on the right. The liver was massively enlarged, weighing 5,450 Gm. The stomach and esophagus were displaced to the left.

CASE 22.—A 27 year old white man was admitted because of recurrent episodes of hemoptysis and pain in the lumbosacral area, and died seventeen hours after admission. Two years previously he had a chancre, for which he received ade-

quate treatment, and subsequently the blood Wassermann test gave negative results. He took several doses of colchicine for rheumatic pains. He was pale, with a moderate icteric tint to the skin. A few inconstant rales were heard throughout both lungs. The liver was slightly enlarged. There were no other findings of note. Shortly after admission his lungs began to bleed actively and, following a massive hemoptysis, he died.

At autopsy both lungs showed extensive metastases which, because of central grayish zones and peripherally placed hemorrhagic areas suggested a diagnosis of choriocarcinoma of the testicle. The primary tumor was found on section of the left testicle, and the tumor mass measured only 2.5 cm. in diameter (fig. 2).

CASE 23.—A 55 year old white man gave a history of severe colicky abdominal pain and vomiting of five hours' duration. Three years previously he noted epigastric pain. During the past six months he had suffered from anorexia, weakness and epigastric discomfort. During the past three months he had noted a slight cough with blood streaking of the sputum on three occasions. On admission he was acutely ill with no significant findings other than those of general peritonitis. Immediate laparotomy revealed general peritonitis originating from a perforation of the proximal portion of the ileum at the site of an intrinsic tumor mass. The resected portion of intestine showed four distinct tumor nodules with an ulcer over each mass. The largest measured 4 cm. in diameter, and at its site there was marked diminution of the lumen. A minute perforation through the serosa was noted. The regional lymph nodes showed metastatic tumor tissue. Several prominent pathologists disagreed as to the diagnosis on study of the resected specimen, their views varying from ganglioneuroma, myosarcoma, lymphosarcoma and metastatic carcinoma or melanoma and malignant carcinoid. The patient's condition gradually grew worse, with death occurring on the fifteenth postoperative day.

At autopsy there were innumerable submucosal and mucosal tumor nodules beginning in the pyloric region and extending down to the distal portion of the sigmoid colon. Most of these presented central ulceration. The bronchus to the lower lobe of the right lung showed an area of narrowing 6 cm. from its origin, with opaque, grayish white tumor tissues replacing the usual bronchial markings and showing limited extension to the regional pulmonary parenchyma.

CASE 24.—A 63 year old white woman entered the hospital because of swelling of the right inferior extremity of three days' duration. The right leg and thigh were markedly swollen. An indurated right fornix and an enlarged mass in the left ovary were found. In the rectum there was also a firm mass which appeared to be beneath the mucosa. The patient went rapidly downhill, showed impending gangrene of the right inferior extremity and died within a week.

Autopsy revealed thrombosis of the right common and external iliac veins. Both ovaries were enlarged, the right measuring 8 by 5 by 3 cm. and the left 6 by 4 by 2.5 cm. On section, both ovaries were occupied by homogeneous grayish white tissue, suggesting Krukenberg tumors. The entire stomach was shrunken and indurated, and on section opaque, firm, grayish white tissue was noted infiltrating the mucosa and submucosa and penetrating through the muscular coat. The inferior vena cava was filled with a large embolus, measuring 8 cm. in length and 1.5 cm. in diameter.

CASE 25.—A 46 year old white man was admitted because of pain in the right upper quadrant of the abdomen and in the paraumbilical region of five weeks' duration. The abdominal pain radiated around the right costal margin to the tip of the right scapula. There was intermittent fever. No other abdominal or thoracic symptoms were noted. On admission, a large mass was revealed in the epigastrium, and there were diminished breath sounds and rales over the upper lobe of the left lung and the lower lobe of the right lung. While the

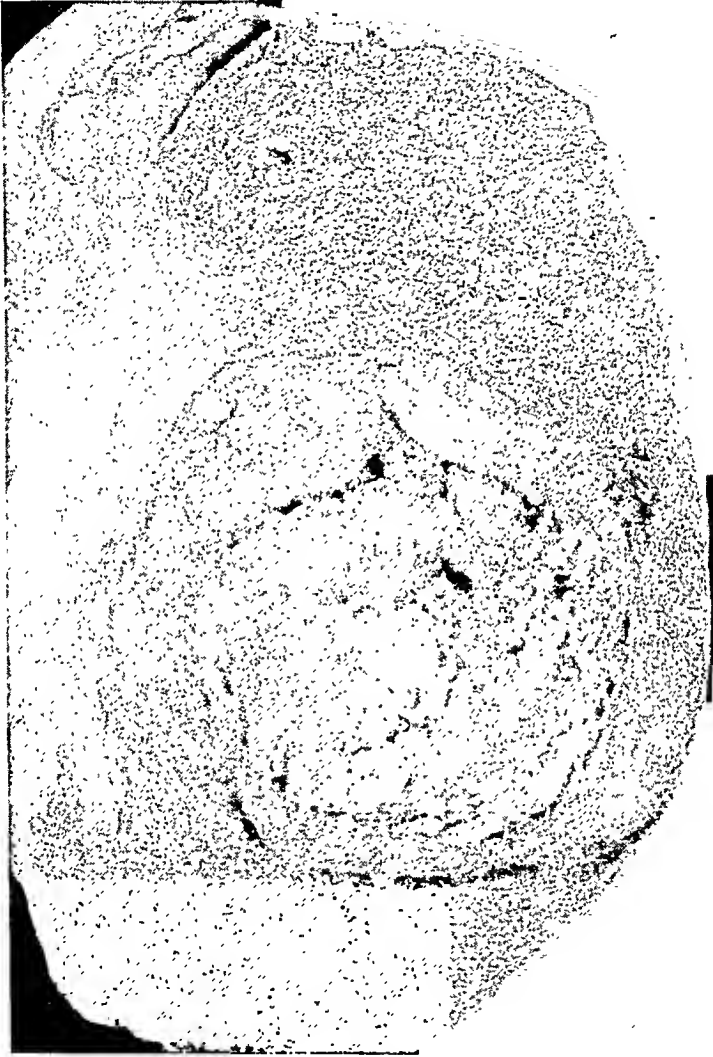


Fig. 2 (case 22).—Left testicle showing replacement of approximately one half of the parenchyma by a primary tumor, diagnosed as choriocarcinoma, without enlargement or distortion of the contour of the organ;  $\times 16$ .

patient was in the hospital there developed on the outer aspect of the left tibia an erythematous indurated area, which became tender and fluctuant. This was at the site of a compound fracture that occurred eight years previously. Incision and drainage were performed, and the material removed was found to be anaplastic neoplasm suggesting sarcoma. Roentgen examination of the chest demonstrated a large, poorly demarcated area of increased density extending outward

from the left hilus. The patient gradually declined and died seven weeks after coming to the hospital.

At autopsy a huge mass was found replacing the right adrenal gland, with marked regional compression of the right kidney. An ulcerating tumor was found in the bronchus to the lower lobe of the left lung (figs. 3 and 4).

In summary, 17 men and 8 women are represented. The ages varied from 27 to 68, with a mean age of 54. Symptoms due to the primary

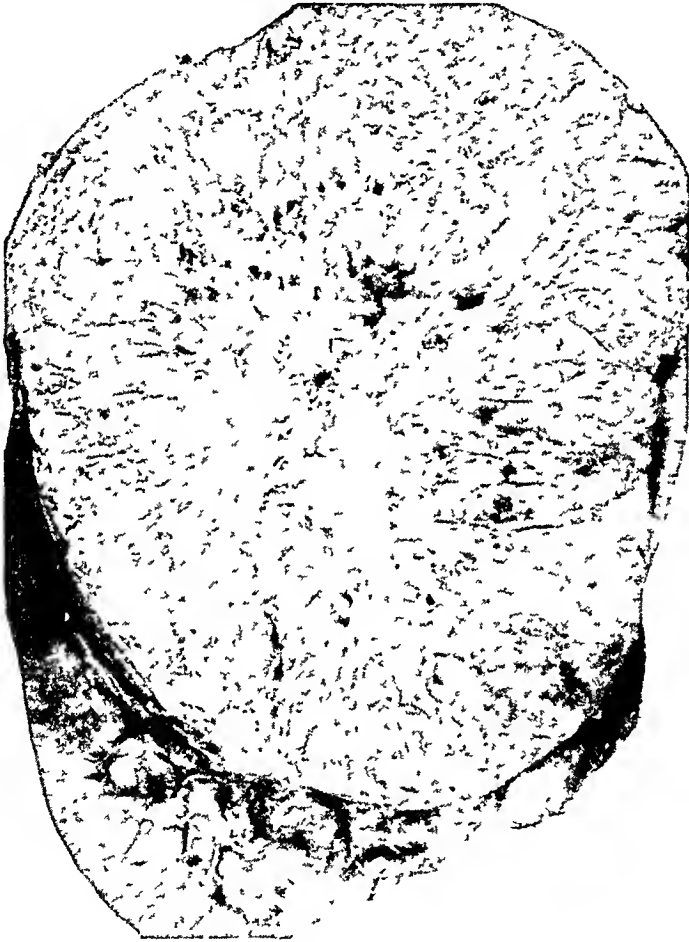


Fig. 3 (case 25).—Massive metastasis to the right adrenal with distortion but no invasion of the underlying kidney

cancer were present before death in 3 cases. In 7 cases general symptoms of cachexia were apparent. A correct diagnosis of the primary tumor was made ante mortem in 3 instances. Although the primary site was suspected in 5 others, it never was definitely established. The primary site was adequately studied clinically in 6 other cases, but the lesion was missed. In 6 instances the thought of cancer was not entertained. In retrospect, a clinical diagnosis of cancer is deemed to have been impossible in 5 cases, while the ability to diagnose the correct

primary site seems to have been remote in 11. In only 10 cases was the primary site accessible for clinical study.

The primary neoplasms varied in size, 12 of them being large and 2 very small. Nine of the tumors showed grade 4 anaplasia. A marked scirrhous reaction was present in 7 instances. Local lymphatic permeation was noted in 13 cases, while invasion of blood vessels in the primary focus was seen on 6 occasions. In the metastatic foci the growth was either large or diffusely invasive. Metastasis throughout the body was found in only 1 case, while metastasis to a distant point only was seen in but 3 cases. Combined local and distant metastases were



Fig. 4 (case 25).—Primary ulcerating carcinoma of the bronchus to the lower lobe of the left lung.

observed in 20 instances. The metastases were lymph borne in 19 and blood borne in 11 instances. Carcinoma was the principal cause of death in all patients with the exception of the 3 in whom massive pulmonary embolism was the immediate terminal event.

The cases listed, while representative, do not include many known and common forms of latent tumor. Willis, in the chapter devoted to this subject, has culled the vast literature of isolated case reports and has indicated the common sites for precocious metastases. Metastatic disease of the cervical lymph nodes has often been found as the first indication of a primary tumor in the nasopharynx, bronchus, larynx, esophagus, stomach or pancreas. Bone foci can be the secondary sites

that call attention to the primary sites of many cancers, among which the thyroid, breast, stomach, lung, kidney and prostate are common epithelial foci of origin. Bone involvement may occasion pathologic fracture or myelophthistic anemia. The central nervous system is another common site for dramatic initial symptoms of a cancer originating from a bronchus, the stomach, a kidney, the pancreas or the thyroid. Initial manifestations due to extension to the peritoneum from the ovary are frequent. Distant hematogenous metastasis to mucous membranes, such as the vaginal, or to the skin is found in cases of hypernephroma, melanoma, choriocarcinoma or bronchiogenic cancer. Such clinical cases have been encountered, but none came to autopsy during the five year period and have been excluded. Any primary cancer can be, in fact, the occasional source of a symptomatic metastasis while presenting no symptoms of itself.

TABLE 2.—*Incidence of Latency*

Primary Site	Cases of Latent Carcinoma	All Autopsy Cases of Carcinoma by Site	All Operative Cases of Carcinoma by Site
Lung.....	8	—61	59
Stomach.....	4	58	25
Pancreas.....	4	38	7
Fundus uteri.....	1	4	8
Prostate.....	2	26	50
Testis.....	1	4	15
Kidney.....	1	3	14
Male breast.....	1	1	2
Ovary.....	1	8	38
Undetermined.....	2	8	..
Total.....	25	211	218

*Incidence.*—During the five year period included in this study, 2,514 autopsies were made, and 389 cases of cancer were found, with a total of 383 individual primary sites. In 8 instances the primary site was never established at autopsy. In 2 instances there were double primary tumors, the rectum being involved in combination with the stomach and the colon, respectively. All cases of miniature asymptomatic incidental adenocarcinoma of the prostate<sup>3</sup> have been discussed in a previous publication and are excluded. Table 2 lists the primary sites and the relative autopsy and surgical incidence of latent forms of cancer. Using the criteria proposed in the discussion of the definition of latency, we found 25 cases of latent carcinoma in a total of 391 cases of carcinoma. These latent primary cancers were found at nine separate sites, corresponding to a total of 203 instances of carcinoma for these sites—an incidence of latency of 11.5 per cent.\* For the total group of 391

3. Baron, E., and Angrist, A.: Arch. Path. 32:787, 1941.

cases of cancer, this represents an incidence of latency of 5.9 per cent. The high incidence of bronchiogenic carcinoma in both the "total" and the "latency" groups is worthy of note. The frequency of diagnoses in the surgical material is seen to parallel the autopsy figures with the notable exceptions of prostatic and ovarian carcinoma. On this basis the clinical and the autopsy aspects of this problem may be said to be parallel.

The sites of the precocious metastases are indicated along with the primary sites initiating the metastatic processes in table 3. The metastases in the central nervous system occasioned manifestations of obstructive hydrocephalus, hemiplegia, the cerebellar syndrome, increased intracranial pressure due to an expanding lesion, and paraplegia associated with extradural spinal involvement. Bone involvement was evidenced by either pain or pathologic fracture. Involvement of the peritoneum and of the pleura usually caused effusion, with pain or intestinal obstruction appearing in two cases. The size of the metastasis presented

TABLE 3.—*Metastatic Foci and Corresponding Primary Sites*

Site of Metastatic Foci	Cases	Site of Primary Carcinoma
Central nervous system.....	5	Lung (2); prostate; stomach; fundus uteri
Long bones.....	4	Lung; stomach; kidney; pancreas
Peritoneum and pleura.....	7	Pancreas (2); ovary; prostate; lung; undetermined; male breast
Lymph nodes.....	3	Pancreas; stomach; undetermined
Liver.....	2	Lung (2)
Lung.....	1	Testis
Intestine.....	1	Lung
Ovary.....	1	Stomach
Adrenal.....	1	Lung

the outstanding feature in 5 instances; in 2 of these the liver was the site of metastasis and in 2 the lymph nodes. The remaining large mass was found in the adrenal (fig. 3). The clinical picture, the precocious metastasis and the primary site are listed in table 3 for purposes of correlation and illustration of latency.

*Factors of Latency.*—The latency of any cancer is determined by its biologic growth processes and by chance. All tumors are asymptomatic in their earliest stages of growth. In general, tumors produce symptoms because of the complications of ulceration, hemorrhage, infection, effusion, necrosis, stenosis or compression. One or many of these complications may occur either with the primary tumor or with the metastasis. The early development of hemorrhage in the secondary site, as in case 22, or suppuration, as in case 5, before similar changes take place in the primary lesion may establish the basis for a diagnosis of latency. In case 23, intestinal obstruction was caused by a bulky ulcerating metastasis in the walls of the ileum. In case 10, the secondary scirrhous peritoneal involvement produced a stenosing lesion of the small intestine, with obstruction. Compression of a vital structure such

as the spinal cord, as in case 3, may patently occasion early complaints. Pleural effusion and ascites as the presenting symptom of a metastasis from a distant focus were seen in cases 14 and 15.

It is obvious that the location of a metastasis of itself will condition the early or late appearance of clinical symptoms. Thus, a lesion in the leptomeninges around the foramens of Magendie and Luschka, as in case 1, or in the region of the internal capsule, as in case 4, will lead to an earlier and more dramatic episode than a lesion of similar size in the occipital lobe. In similar fashion, a hepatic metastasis (cases 20 and 21), though large, will lie dormant for a greater length of time than a bone lesion of like dimensions.

The proximity of the tumor to large vascular structures, as in case 22, will be determinative of catastrophic symptoms as compared with an equivalent lesion in the relatively avascular periphery of an organ. Location in the tail of the pancreas (cases 10, 13 and 18), in the periphery of the lung (case 20), in the periphery of the kidney (case 6), or in the fundus of the stomach (case 8) will tend to keep the primary lesion silent. The inaccessibility of tumors of internal viscera and the contrasting accessibility of their metastatic foci predisposes to the evolution of the syndrome of latency.

Some inherent biologic properties of the individual neoplasms play a role in determining their latency. The rate of growth in the metastasis as compared with that at the primary site is one such factor. The known tendency of certain neoplasms to invade vascular channels locally and to metastasize to distant sites is well illustrated by the small choriocarcinoma of the testis in case 22 and by the hypernephroma in case 6. Early lymphatic metastasis plays a similar role, as seen in cases 15 and 16.

As will be noted in table 2, the lung, the stomach and the pancreas are the common primary sites in the latent material, as well as the major sites for the distribution of carcinoma in the whole autopsy series. That the frequency of occurrence of tumors of itself does not exclusively determine the frequency of latency is borne out by the fact that in this same autopsy series carcinoma was found in the rectum thirty-nine times and in the sigmoid twenty-four times, while the bladder, the esophagus and the colon were represented by 21, 17 and 12 such lesions, respectively; yet no latent cancers were encountered in any of these primary sites. The series of cases of latent primary carcinoma is a small one, but it is noteworthy that the incidence of latent carcinoma in the lung and in the pancreas is particularly out of proportion to the general incidence of carcinoma in the total group of autopsies.

Many common examples of latent cancer are not included in this series, as noted. It is hoped that the analysis of the term "latency"



and the establishment of specific limitations for its application will prompt others to report larger series, so that the true incidence of latent primary cancer can be rendered available to the clinician and the pathologist.

#### SUMMARY

An analysis and definition of the term "latency" in regard to cancer are offered. It is suggested that the term be used only for cancer which produces symptomatic precocious metastases while itself remaining silent.

Twenty-five illustrative cases of carcinoma meeting the limitations of this definition were encountered in a series of 2,514 autopsies covering a five year period, an incidence of 5.9 per cent of all cases of carcinoma.

The main factor contributing to latency is inaccessibility of the primary cancer, coupled with the tendency to produce early massive or strategically located metastases.

# HISTOLOGIC ANALOGY OF BRONCHIAL ADENOMA TO LATE PRENATAL AND EARLY POSTNATAL STRUCTURES

WILLIAM H. HARRIS JR., M.D.

NEW ORLEANS

Only in recent years has emphasis been placed on the subject of noncancerous tumors of the bronchial tree. Of these tumors, "adenoma" is the most important. Womack and Graham<sup>1</sup> expressed the opinion that tumors of this group are common. Goldman and Stephens<sup>2</sup> stated that such tumors comprise 6 to 10 per cent of all bronchial tumors and represent 25 per cent of all resectable bronchial tumors. Pollak, Cohen and Grassi<sup>3</sup> in a review of 104 cases of benign bronchial tumor found that 49 per cent were cases of adenoma. Thus, on the basis of incidence alone, adenoma of the bronchial tree assumes importance. This is not the only aspect to be considered, however, for much has been written as to the possible causes and as to the mother tissue.

Several theories of origin have been suggested.

Fried<sup>4</sup> expressed the belief that they arise from bronchial mucous glands.

Reisner<sup>5</sup> and Wessler and Rabin<sup>6</sup> expressed the opinion that they arise from the duct epithelium of bronchial mucous glands. Goldman and Stephens<sup>2</sup> suggested that the epithelium of either the ducts or of the glands may be the site of origin.

According to Laff,<sup>7</sup> Mallory stated that these tumors resemble carcinoids of the intestinal tract.

Womack and Graham<sup>1</sup> called them "mixed tumors" and pointed out their resemblance to fetal lung. They stated further that other anomalies of various kinds are not infrequently associated. They thus favored the conception that these tumors are formed from embryonic

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1. Womack, N. A., and Graham, E. A.: *Arch. Path.* **26**:165, 1938.
2. Goldman, A., and Stephens, H. B.: *J. Thoracic Surg.* **10**:327, 1941.
3. Pollak, B. S.; Cohen, S., and Grassi, A. M.: *Arch. Otolaryng.* **27**:426, 1938.
4. Fried, B. M.: *Arch. Otolaryng.* **20**:375, 1934.
5. Reisner, D.: *Arch. Surg.* **16**:1201, 1928.
6. Wessler, H., and Rabin, C. B.: *Am. J. M. Sc.* **183**:164, 1932.
7. Laff, H. I.: *Arch. Otolaryng.* **31**:148, 1940.



Figure 1

(See legend on opposite page)

bronchial buds that have failed to pursue normal development. The tumors contain both endodermal and mesodermal elements. Jackson and Konzelman<sup>8</sup> indicated that they were not convinced of an origin from the epithelium of mucous glands. In discussing the paper of these authors, Stout and Churchill<sup>9</sup> both expressed the belief that adenoma arises from a vestigial structure of some type, possibly the "mediastinal lobe" of the fetal lung. Harris and Schattenberg<sup>10</sup> in the consideration of various embryonal types of tumors of the lungs, have suggested that tumors of the secretory gland type may arise from "rests" of such glands.

#### HISTOLOGY

In all of the discussions of these tumors from the standpoint of histology their vascularity is emphasized. This feature is described repeatedly.<sup>11</sup> The individual cells are usually stated to have small round darkly staining nuclei with a scanty amount of cytoplasm. It has also been frequently noted that a mistaken diagnosis of endothelioma might readily be made. Edwards and Taylor<sup>11c</sup> reported 4 cases in which the features of blood vessels, lymphocytes, and spindle cells in whorls were so prominent that they called the tumors "vascular endotheliomas." They stated that in a personal communication Barnard drew attention to some resemblance to lymphadenoid tissue. Brunn<sup>11e</sup>

8. Jackson, C. L., and Konzelman, T. W.: *J. Thoracic Surg.* **6**:312, 1937.

9. Stout and Churchill, in discussion on Jackson and Konzelman.<sup>8</sup>

10. Harris, W. H., and Schattenberg, H. J.: *New Orleans M. & S. J.* **94**:333, 1942.

11. (a) Zamora, A. M., and Schuster, N.: *J. Laryng. & Otol.* **52**:337, 1937. (b) Brunn, H., and Goldman, A.: *Surg., Gynec. & Obst.* **71**:703, 1940. (c) Edwards, A. T., and Taylor, A. B.: *Brit. J. Surg.* **25**:487, 1938. (d) Goldman, A.: *California & West. Med.* **53**:123, 1940. (e) Brunn, H.: *J. Thoracic Surg.* **9**:119, 1939. (f) Womack and Graham.<sup>1</sup> (g) Fried.<sup>4</sup> (h) Laff.<sup>7</sup> (i) Jackson and Konzelman.<sup>8</sup>

#### EXPLANATION OF FIGURE 1

*A*, photomicrograph showing the general topography of the sections studied. Lymphoid nodules are indicated by arrows; *A* designates the aorta; *E*, the esophagus; *B*, the divisions of the bronchi.  $\times 10$ .

*B*, photomicrograph of bronchial mucous glands of a newborn infant, showing rather ill defined cell membranes and a somewhat haphazard arrangement of acini within the limiting basement membrane.  $\times 200$ .

*C*, photomicrograph of bronchial mucous glands of a newborn infant at lower magnification, to show the relationship of these glands to the lumen (*L*) and the cartilaginous rings of the bronchi.  $\times 50$ .

likewise commented on the resemblance of the tumor cells to lymphocytes in his cases.

Other forms of adenoma, however, show a histologic structure which does have a secretory gland appearance, the main body of the tumor being made up of polyhedral cells with large round or oval reticular nuclei arranged in acini or in solid columns.<sup>7</sup> In still other forms there is a definite mixture of the glandular type cells and the cells with small round dark nuclei. Thus it certainly seems likely that there are here two possible elements: (1) mesodermal, showing especially cells resembling lymphoid tissue with varying proportions of blood vascular elements; (2) endodermal, showing glandular cells resembling the glandular and ductal "epithelium" of the bronchial mucous glands.

These statements are in accord with the work of Womack and Graham<sup>1</sup> and would support their use of the term "mixed tumors."

#### MATERIAL STUDIED

There were available to me a considerable number of fetuses and newborn and stillborn infants. Since mention had been made of the possibility that adenoma is related to embryonic and fetal tissues, a study of the material from this point of view was undertaken.

Fetuses ranging in age from 7 months to maturity and infants who died at from a few hours to three months after birth were studied—23 in all. In each instance a transverse section through the trachea and surrounding structures, just above the bifurcation, was prepared. A similar section was obtained through the region of the stem bronchi approximately 1 cm. beyond their origin. These areas were selected because they represent the predominant sites of such neoplasms. Figure 1 *A* reveals in general the topography of the regional structures concerned.

Duplication of findings was so consistent in all the age periods enumerated that general statements applicable to the observations in all specimens may be made.

#### STEM BRONCHI

The salient features of observation were the character and the location of secretory glands. It may be primarily mentioned that the bronchial epithelium at these stages, while of a columnar ciliated type, is of delicate texture, easily disturbed and somewhat pale in tinctorial property. The lining epithelium of the glandular acini is quite at variance with the usual regulated, orderly arrangement of more or less pyramidal cell type present after the third month. The cell membranes are poorly formed, the cell outline is ragged, and the cells are distributed rather haphazardly within the limiting basement membrane (fig. 1 *B*). Occasionally a more immature gland is noted in which merely formative cells are aggregated. This same general aspect of poorly developed glandular elements has been noted in a bronchiogenic tumor (fig. 2 *A*).

Such glandular patterns form a distinct contrast to those noted after maturation (fig. 2 *B*) or in a simple bronchial glandular polyp in which proper configuration of cells and arrangement are present.



Fig. 2.—*A*, photomicrograph of a bronchial "adenoma" showing predominant glandular elements, rather poorly developed and resembling the bronchial mucous glands of the newborn infant (fig. 1 *B*).  $\times 250$ . *B*, photomicrograph of normal bronchial mucous glands of an adult. The more mature acinous arrangement, with nuclei at the base of the glandular cells, is to be noted.  $\times 200$ .

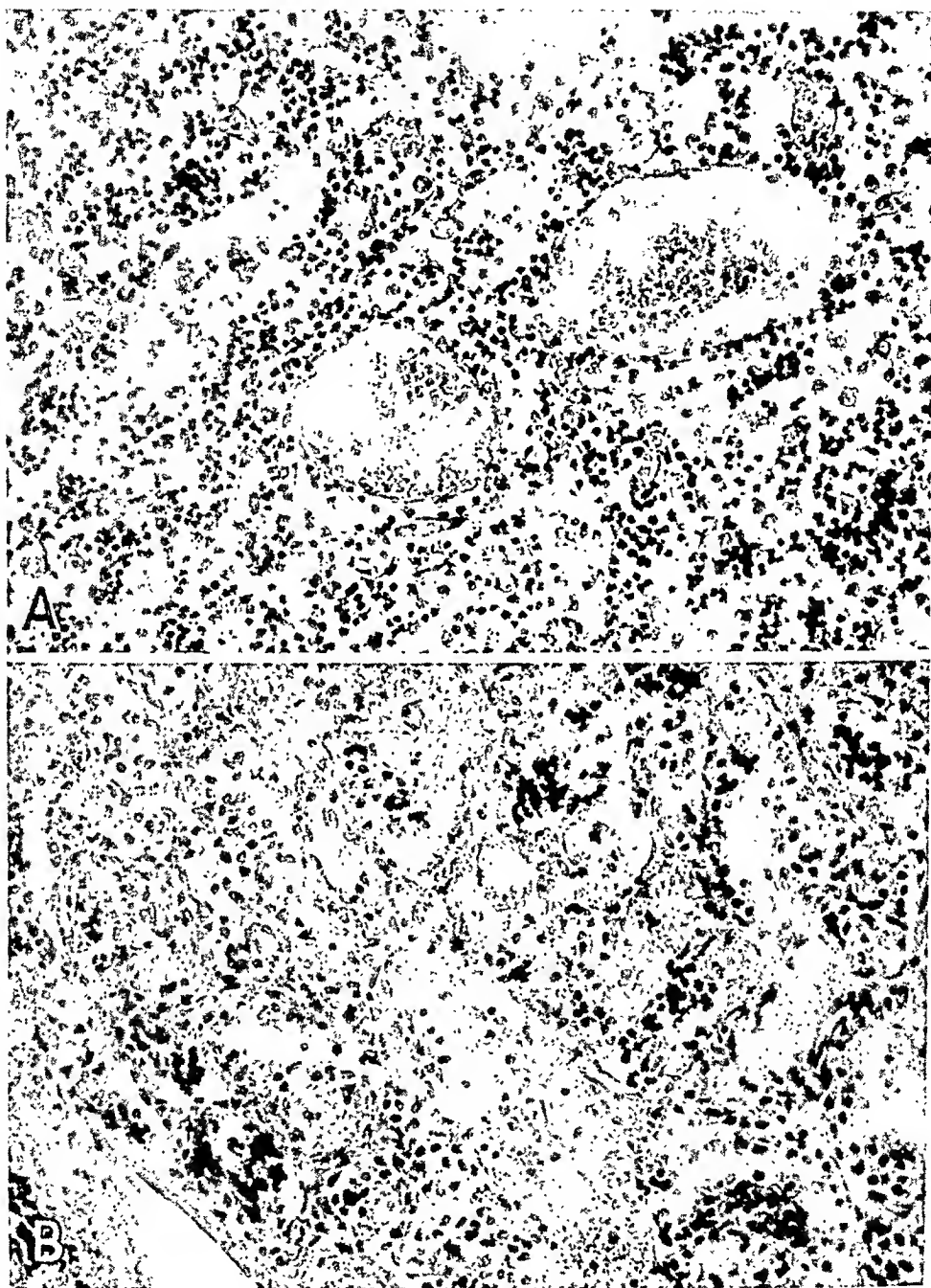


Fig. 3.—*A*, photomicrograph of bronchial lymphoid tissue of a newborn infant, showing the absence of germinal centers, the loosely arranged stroma and the very large and prominent sinuses containing many red blood cells. Other red blood cells are scattered among the lymphoid elements.  $\times 200$ . *B*, photomicrograph of bronchial "adenoma" showing the predominant cells with small round darkly staining nuclei, resembling lymphocytes. Large sinusoidal spaces containing many red blood cells are also seen. Other red blood cells are dispersed through the section. Note the resemblance to *A*.  $\times 200$ .

## LYMPHOID AND VASCULAR STRUCTURES

The vascular structures of the bronchi consist of numerous thin-walled blood vessels in which the mural development is greatly restricted. They appear more like blood sinuses than true vessels. They are of various sizes and are distributed to the greater extent in the submucosa. Such characteristics may, however, persist at times in adult structure. What is especially noteworthy is that these blood vascular characteristics are greatly exaggerated in the closely approximated and often contiguous lymphoid tissue. Not only are such blood sinuses very numerous and diffusely scattered throughout, but the blood cells and lymphoid cells may be intimately dispersed without evidence of mural separation. The stroma of these immature lymphoid glands is slight and irregularly arranged. Furthermore, there is no cortical and medullary differentiation or nodule formation. The germinal centers have not developed at such stages (fig. 3 *A*). It is to be stressed that these structures are in contiguous juxtaposition to the outer structure of the main stem bronchi and at times may protrude into the walls of the bronchi. This feature is emphasized in that bronchiogenic adenoma may closely simulate both the cellular pattern and the vascularity of such lymphoid developments (fig. 3 *B*). In the tumor formation, however, a delicate stromal structure is often arranged in acinal compartments.

## COMMENT

These observations deal with the features of immaturity in very early life of certain structures of the main stem bronchi and the adjacent lymphoid tissue. The embryonal malplacements or maldevelopments of such structures have not been considered here. However, the intricacies of developmental characteristics of the lungs have been stressed by Gruenfeld and Gray,<sup>12</sup> wherefrom it can be deduced that these structures are likely fields for such complications. That such actually occur was shown by Flint,<sup>13</sup> who described the occurrence of squamous cell islands in the lining of normal bronchi, and by Rector and Connerly,<sup>14</sup> who emphasized the presence of aberrant islands of columnar ciliated epithelium and of gastric mucosa in the esophageal lining.

Neglecting these possibilities, however, the observations reported here simply point out the similarity of infantile types of structures, especially the bronchial mucous glands and the peribronchial and peritracheal lymphadenoid tissue, to the histologic findings in bronchial adenoma. Since, in the material studied here, the bronchial mucous glands occurred not only just beneath the mucosa but more deeply

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12. Gruenfeld, G. E., and Gray, S. H.: *Arch. Path.* **31**:392, 1941.

13. Flint, J. M.: *Am. J. Anat.* **6**:1, 1906.

14. Rector, L. E., and Connerly, M. L.: *Arch. Path.* **31**:285, 1941.



between the cartilaginous rings, and since the lymphadenoid tissue was found not only peribronchially and peritracheally but within the walls of bronchi and trachea as well, it can be deduced that tumors arising from such tissues may be intramural or extramural or may extend into the bronchial or the tracheal lumen. Such locations for bronchial adenoma have been described by Goldman.<sup>11d</sup> It is also to be mentioned that adenomatous tumors have been found to occur in early life and often date their inception within the first decade of life.

The potentiality of these immature histologic structures for originating or assuming cancerous characteristics is, of course, conceivable.

The present observations are compatible with those of Womack and Graham<sup>1</sup> and the subsequent ones of Harris and Schattenberg.<sup>10</sup> The general term "adenoma" does not seem technically correct for all of these tumors, some being predominantly lymphoid in appearance. Likewise, since all are not of both lymphoid and secretory gland structure, the term "mixed tumor" is not always applicable. Perhaps an added suffix, such as "secretory type," "lymphoid or angiolymphoid type," or "mixed type," would make the appellation "adenoma" more comprehensive.

# ACROMEGALY WITH LONG-STANDING TUMOR INFILTRATION OF THE CAVERNOUS SINUSES

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Since Marie's<sup>1</sup> classic description of acromegaly in 1886 many hundreds of cases have been reported with detailed anatomic studies.<sup>2</sup> The many advances of the last decade in knowledge of the function of the hypophysis have given a more rational interpretation of its numerous and complex morphologic changes. New concepts regarding cell lineage in the anterior lobe (Severinghaus<sup>3</sup>) and an understanding of the many factors capable of altering the structure and function of the pituitary gland have aided considerably in clarifying the older and somewhat confusing morphologic studies of acromegaly, as well as those of other pituitary diseases.

The case of acromegaly to be described is one of those in which unusual and atypical features are exhibited which would be confusing if viewed solely from their anatomic aspects but which can be reasonably explained from the physiologic point of view.

## REPORT OF A CASE

A man 27 years of age was admitted to Montefiore Hospital in November 1915. The only relevant point in the family history was that all members of his family were large and stocky. His illness began in 1912 with severe occipital headaches radiating down both shoulders. After two years the pains radiated to the face and were dull and continuous, with frequent exacerbations. In 1911 he married, and his wife bore two healthy children. His libido and potency were excessive for two years, but beginning in 1913 they progressively diminished, and

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From the Laboratory Division of the Montefiore Hospital.

1. Marie, P.: *Rev. de méd.*, Paris **6**:297, 1886.

2. (a) Atkinson, F. R. B.: *Acromegaly*, London, John Bale Sons & Daniels-son, Ltd., 1932; (b) *Endokrinologie* **17**:308, 1936. (c) Berblinger, W.: *Pathologie und pathologische Morphologie der Hypophyse des Menschen*, in Hirsch, M.: *Handbuch der inneren Sekretion*, Leipzig, 1932, vol. 1, pt. 6, pp. 910-1097. (d) Cushing, H., and Davidoff, L. M.: *The Pathological Findings in Four Autopsied Cases of Acromegaly, with a Discussion of Their Significance*, Monograph 22, Rockefeller Institute for Medical Research, 1927.

3. Severinghaus, A. E.: *A. Research Nerv. & Ment. Dis.*, Proc. (1936) **17**: 69, 1938.

by March 1915 he became completely impotent. In 1914 he began to suffer from polydipsia, polyphagia and polyuria, and his weight dropped from 180 to 153 pounds (81.5 to 69.5 Kg.). In 1911 he wore shoes of size 9, while by 1915 his shoes were of size 10 or 11. In 1915 he began to suffer pains in the joints of his hands and knees and became progressively weaker. At this time (early in 1915) a change in his features was noted. There was an increase in hair growth from the onset of his illness.

He was admitted to the Mount Sinai Hospital in New York in May 1915. There typical acromegalic features were noted, and large hands with the fingers thickened throughout their length. The left lobe of the thyroid was enlarged, and there was a definite exophthalmos. The Stellwag sign was present. Three incisors of the lower jaw were separated, loose and carious. The rest of the teeth were in fairly good condition. The roentgenogram of the skull revealed large sinuses but a small sella turcica, with the anterior and posterior clinoid processes almost meeting. The eyegrounds were normal. Lumbar puncture revealed an initial spinal fluid pressure of 240 mm. of water. The urine contained less than 0.1 per cent reducing bodies.

On his first admission to Montefiore Hospital, in November 1915, the patient was 73 inches (185.5 cm.) in height and weighed 153 pounds (69.5 Kg.). The features were typically acromegalic. The hard palate was high and narrow. The thyroid gland was enlarged, and the external genitalia were larger than normal. The heart and lungs were normal. Muscle power was diminished. The skin of the body was moist and warm, while that of the toes was cold and cyanotic. A few verrucae were present. The hair was thick and dense on the head, the eyebrows and the pubis. The left palpebral fissure was smaller than the right, but the pupils were equal and reacted to light and in accommodation. The urine contained reducing bodies up to 5 per cent. The hemoglobin content was 85 per cent with 4,200,000 red blood cells.

Neurologic examination December 13 revealed normal ocular movements and normal visual fields. The optic disks showed no evidence of neuritis. However, it was observed that the veins of the upper eyelids were dilated.

The facial pains were severe and were described by the patient as "pins and needles." The headaches continued unabated and were now chiefly supraorbital.

Feb. 10, 1916, weakness in the lower part of the right side of the face was observed.

March 30 Dr. C. Elsberg performed a craniotomy on the right temporal area. The intracranial pressure appeared normal, and no abnormalities were noted. The wound was therefore closed without any other surgical maneuver. Following the operation, the patient manifested symptoms and signs referable to the cranial nerves. Three days after operation the patient complained that he was unable to see on the left side, and examination revealed left homonymous hemianopia. The next day the defect in the visual field diminished to a quadrantic area on the left side. April 9 the patient was able to see objects close to him, but they appeared blurred. There were no signs of optic neuritis, and the disks were pale. April 10 the patient complained of double vision. Examination at this time revealed marked limitation of motion of the left internal and superior recti muscles and incomplete ptosis on the left. Diplopia was present only at close range and a little to the right of the median line.

Neurologic examination April 13 revealed bitemporal hemianopia with the left nasal field being less extensive than the right. The field of vision was thus restricted more in the left eye. Both optic disks showed decided pallor, the left more than the right. The left pupil was somewhat larger and slower in response

to strong light stimulation than the right. There was diplopia in the left nasal field of vision. There was intense pain on test movements or on looking intently. Improvement in vision gradually occurred and by April 28 vision was 20/20 in the right eye and 20/30 in the left eye.

Two months after the operation the patient showed marked improvement. The headaches disappeared, and there were no visual disturbances aside from slight myopia. There was no longer any glycosuria even on a regular house diet. Muscular strength increased. Sexual vigor returned but lasted only three months. The extreme sensitiveness of the bones of the skull to slight pressure that had existed before the operation entirely disappeared. At the time of discharge there was only an occasional pain in both the supraorbital and the suboccipital region. There were no longer any pains in the face. The only remaining sign of involvement of a cranial nerve at the time of discharge was slight weakness of the lower part of the right side of the face. During his ten month stay in the hospital the patient gained 22 pounds (10 Kg.).

Following his discharge from Montefiore Hospital, he worked and gained weight up to 205 pounds (93 Kg.). He remained in comparatively good health for fifteen years. In August 1931 he was struck by an automobile and rendered unconscious for a few minutes. A diagnosis of contusion of the head was made at Bellevue Hospital, New York. During his three day stay at that hospital glycosuria was discovered. After the accident he suffered intermittent frontal and occipital headaches and glycosuria remained.

He was readmitted to Montefiore Hospital in April 1932, complaining of cough, pain in the left side of the chest, loss of weight and general weakness. Examination revealed physical and roentgenographic signs of consolidation of the upper lobe of the left lung. The sputum contained numerous acid-fast bacilli. Roentgenograms of the skull (fig. 1A) revealed no evidence of destruction of the floor of the sella or of the clinoid processes. The blood pressure was 120 systolic and 80 diastolic. The body weight was now only 177 pounds (80 Kg.). The basal metabolic rate was  $+1$  and  $+7$  per cent. The urine contained 1 to 5 per cent dextrose, and the fasting blood sugar amounted to 323 mg. per hundred cubic centimeters. The hemoglobin content was 63 per cent, and the red blood cell count was 3,600,000.

The jaws were now completely edentulous, and the tongue was large. The body musculature was flabby and hypotonic. The eyegrounds showed no changes. The visual fields were roughly normal, but the right temporal field was slightly less extensive than the left. The right palpebral fissure was wider than the left, and the pupils were small, slightly irregular and showed a poor reaction to light. The movements of the eyeballs were normal. The left nasolabial fold was more prominent than the right. Of interest is the fact that the patient sneezed when light was flashed in either eye.

The diabetes was moderately severe, and the pulmonary lesion was active. Pneumothorax was induced on the left side. With dietary control and insulin (80 units daily) the diabetes and the tuberculosis both improved. By March 1933 insulin was no longer necessary. He was discharged to the tuberculosis clinic in September 1933. Insulin was again necessary in June 1935.

In August 1935 the patient began to suffer recurrent anginal attacks, which were unrelieved by nitrites or sedatives. An electrocardiogram made in August 1935 was normal, but one made four months later showed left axis deviation and some slurring of the QRS complex in lead III.

Two days before his last admission, July 30, 1936, he suffered nausea and took neither food nor insulin. The day of admission he suffered epigastric pain

which radiated to the substernal region. Four hours after admission to the hospital he passed into a state of peripheral circulatory collapse and died within two hours. The diagnosis of acute coronary occlusion was made. The autopsy was performed fourteen hours post mortem.

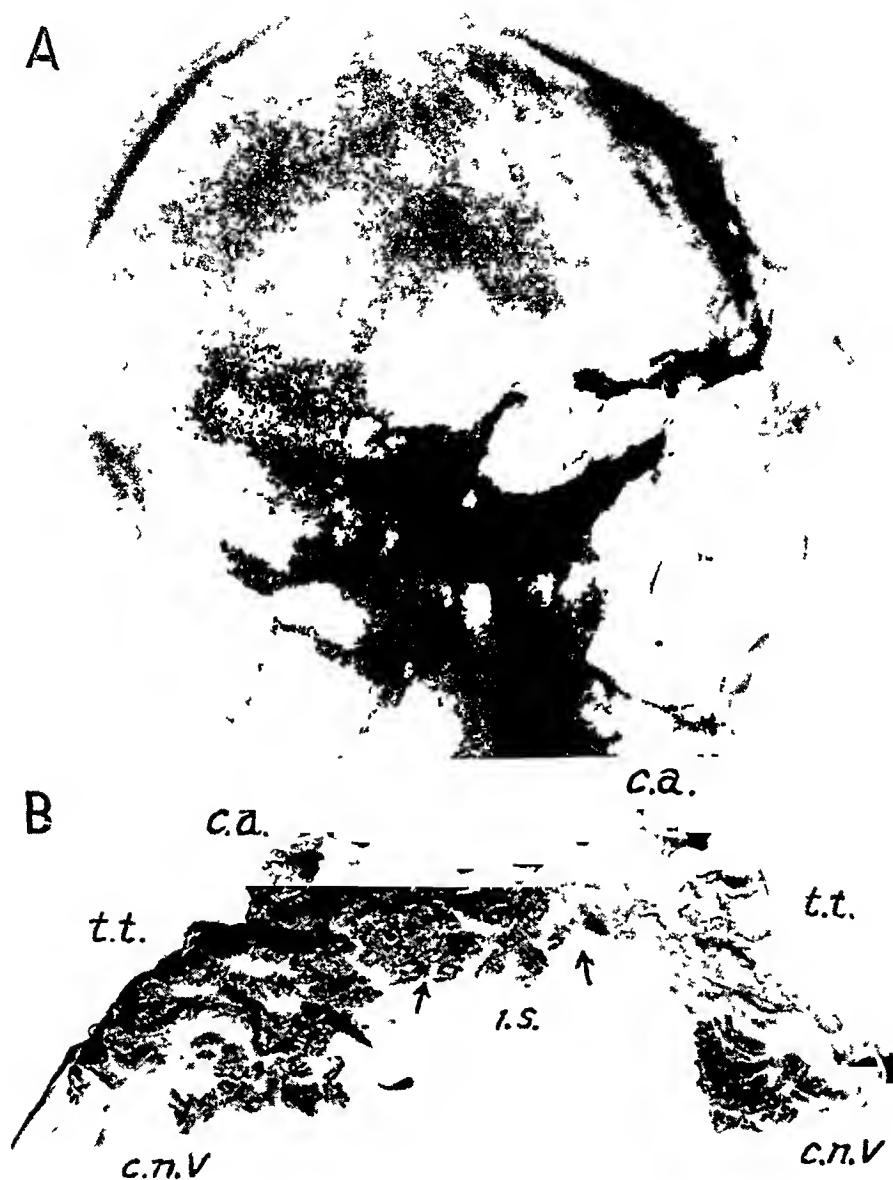


Fig 1.—*A*, lateral roentgenogram of the skull. Note the bony defect at the site of the temporal craniotomy, the normal-sized sella turcica and the huge paranasal sinuses. *B*, dorsal view of the hypophysis and of the contents of the cavernous sinuses (approximately  $\times 22$ ): *ca*, carotid artery; *i.s.*, infundibular stalk; *c.n.v.*, filaments of trigeminal nerve roots; *t.t.*, tumor tissue in the cavernous sinuses. Arrows delimit the lateral borders of the hypophysis.

*Autopsy.*—The features were typically acromegalic. The fingers were thick and broad. The skin was thick and tough and that on the face and back was covered with numerous small fibrous verrucae. The body was 74 inches (188 cm.)

long. The liver edge was 4 fingerbreadths below the right costal margin. Both lungs were attached to the wall of the chest by numerous firm adhesions. Each pleural space contained about 300 cc. of clear straw-colored fluid. The pericardial cavity contained about 30 cc. of clear fluid.

The heart weighed 550 Gm. It was dilated and hypertrophied. The left ventricle measured 22 mm., and the right ventricle 5 mm., in thickness. The myocardium was firm and of normal color. There were no gross areas of scarring. The valves showed no abnormalities. The right auricle was dilated. Each coronary artery showed marked atherosclerosis throughout its course. In places the lumen was reduced to a pinpoint opening. No thrombi were noted in the larger branches. The aorta presented marked atherosclerosis with many ulcerations.

The right lung showed congestion and edema. The pleura of the left lung was thickened. On section of this lung there were two cavities in the upper lobe filled with yellowish green purulent material. Scattered throughout both lobes were numerous acinonodular tuberculous nodules, some of which were calcified.

The liver weighed 3,300 Gm. and was considerably enlarged. It was firm with a sharp edge. The gross structural configuration was normal.

The gallbladder presented no abnormalities.

The spleen weighed 450 Gm. The capsule was smooth. On section the pulp was soft and scraped away easily.

The pancreas weighed 180 Gm.

The right adrenal weighed 10 Gm. and the left 13 Gm. On section the cut surface was yellow. The medulla showed some autolysis. No nodules were observed grossly.

The right kidney weighed 410 Gm. At the upper pole was a large solitary cyst, 5 by 5 cm. The left kidney weighed 400 Gm. The pelvis was slightly dilated. In the bladder slight congestion of the trigone was noted.

The prostate was enlarged. In the left lobe a large abscess extended into the seminal vesicle and the vas deferens. The right lobe was normal. Both testes were normal in size and shape. The tubules strung out easily. The left epididymis was enlarged and firm, and showed numerous yellowish cheesy nodules on section.

The stomach was moderately dilated. The measured capacity was 4,000 cc. The small intestine was normal. In the colon at the hepatic flexure was a rounded, irregular, well circumscribed, raised tumor mass, 5 by 5 cm. The remainder of the bowel was normal.

The lymphatic system showed enlargement of many nodes in the mesentery but no evidences of tuberculosis or tumor grossly.

The thyroid weighed 110 Gm. Both lobes were considerably enlarged. No nodules were present. The cut surface was glassy and amber colored. The fourth parathyroids were slightly enlarged.

A small remnant of the thymus was found.

In the right temporal bone there was a roughly circular surgical defect, about 3 cm. in diameter. The inner surface of the skull showed marked elevations and depressions corresponding to the convolutions of the brain. The skull was particularly thick in the frontal region. The dura was adherent to the underlying right temporal lobe.

The brain weighed 1,360 Gm. The blood vessels at the base showed moderate atherosclerosis. The optic tracts, chiasm and nerves appeared normal.

The infundibular stalk of the hypophysis was unusually stout. The diaphragm of the sella turcica was flat. The dura overlying both cavernous sinuses was

raised from the base of the skull by an infiltration of tissue, which was soft and pinkish yellow on section. This elevation of the dura extended for a distance of 1.5 cm. on each side.

Both cavernous sinuses were completely filled by this tumor tissue as far anteriorly as the sphenoidal fissures and posteriorly to the anatomic limits of the sinuses. The hypophysis itself was small, measuring 10 by 7 by 7 mm., and was attached to, but not fused with, the tumor masses. The carotid arteries, the nerve trunks, the dura and the bone showed no gross evidence of tumor invasion. There were no obvious gross abnormalities of the other venous sinuses. The hypophysis and the adjacent tumor masses were dissected out in toto (fig. 1B).

After removal of the hypophysis and the contents of both cavernous sinuses en masse, it was noted that there was generalized thinning of the sella turcica and of the adjacent greater wings of the sphenoid bone. The anterior and posterior clinoid processes showed only slight absorption. The hypophysial fossa was of normal dimensions, 11 mm. in its anteroposterior diameter and 11 mm. transversely. The nasopharynx was carefully examined for the presence of tumor, but none was found grossly.

*Microscopic Observations.*—The heart muscle fibers were normal to slightly hypertrophied. There were several microscopic areas of recent infarction, i. e., coagulation necrosis of muscle fibers, congestion of the capillaries and polymorphonuclear leukocytic infiltration. A few areas of microscopic scarring and of fatty metamorphosis were present. The myocardial sinusoids were increased in number.

In the lung there were caseating conglomerate tubercles surrounded by an alveolar exudate of fatty phagocytic cells. There was acute congestion of the alveolar capillaries.

The liver showed no congestion. The nuclei of the liver cells were vacuolated. There was lymphocytic infiltration in the portal areas.

In the spleen, the reticulum network was thickened, and there was an infiltration of polymorphonuclear leukocytes in the pulp.

The tumor mass in the colon was typical adenocarcinoma.

The leg muscle fibers were large and the cross striations intact.

The kidney showed slight arteriolosclerosis and arteriosclerosis. The glomeruli disclosed no significant changes. The tubules showed granular degeneration.

The epididymis on the left showed acute purulent inflammation with abscess formation. In several veins there was an infiltration of polymorphonuclear leukocytes and lymphocytes. No tubercles were present.

The acini in the uninfected portion of the prostate showed no abnormalities.

The adrenal cortex was thick and rich in lipid. It showed numerous areas of nodular hyperplasia and several small foci of lymphocytes. The glomerular zone showed slight fibrosis. The medulla disclosed no changes.

In the testis about one third of the tubules showed varying degrees of atrophy up to complete fibrosis. The remainder revealed moderately diminished spermatogenesis. Few adult spermatozoa were present. The stroma was edematous and slightly increased. The basement membrane of the tubules was thickened. Leydig cells were present in average numbers.

In the pancreas the islets of Langerhans were diminished in number but were of good size and of normal structure. Slight arteriolosclerosis and arteriosclerosis were present.

The follicles of the thyroid varied considerably in size from small to large. The epithelium was flat to cuboidal, and the colloid was dense. There was a diffuse increase in the stroma. There was one large nodule with large follicles and cuboidal epithelium.

All 4 parathyroids were removed and sectioned. Two glands were hypertrophied. Water clear cells and oxyphil cells were rare in all the glands. The dark chief cells predominated. There was little lipomatosis. One gland showed an extreme degree of follicle formation with retention of colloid.

In the hypothalamus the ganglion cells did not show the normal amount of iron pigment. Some appeared slightly swollen and disintegrated.

The right optic nerve at the optic foramen had a few minute foci of tumor cells in the outer layers of the nerve sheath.

The contents of the left sphenoidal fissure included several foci of tumor cells, 1 to 2 mm. in diameter. There was no invasion of nerve trunks. The contents of the right sphenoidal fissure showed several microscopic foci of tumor cells. There was no involvement of nerve trunks by tumor cells.

The posterior region of the tumor mass (through the left gasserian ganglion) revealed no infiltration of nerves or of the ganglion. In one area the outer layers of the dural roof of the cavernous sinus contained strands of tumor cells.

In a coronal section through a cavernous sinus and the lateral portion of the hypophysis the carotid artery showed marked atherosclerosis. The adventitia was infiltrated by tumor cells. At one level there was a single layer of tumor cells between the media and the thickened intima, with a large clump of tumor cells in an atheromatous abscess in the intima. The nerve trunks were intact, but there was an infiltration of tumor cells among them. Several minute calcific deposits and microscopic islands of osteoid tissue were present in the tumor tissue.

In a coronal section through the largest diameter of the tumor mass in a cavernous sinus the carotid artery was separated from the nerves in the lateral wall of the sinus by a mass of tumor tissue 12 mm. in thickness. The adventitia and in some areas the outer layers of the media of the carotid artery were infiltrated by tumor cells. There were several follicles filled with colloid up to 0.5 mm. in diameter. No blood spaces were present. The gasserian ganglion showed scattered ganglion cells in various stages of dissolution—nuclear pyknosis and neuronophagia.

In a coronal section through the middle of a cavernous sinus and including the lateral portion of the hypophysis (fig. 2*B*) the carotid artery was separated from the lateral wall of the sinus by tumor tissue only 5 mm. in thickness. There was only one microscopic focus of tumor cells in the region of the nerve trunks. The media of the carotid artery was free from tumor infiltration. The section through the hypophysis apparently was through the region of the pars intermedia, for several colloid-filled cysts were present. The pituitary tissue consisted largely of ripe basophils. A few ripe acidophils, transitional acidophils and transitional basophils were present. In the region adjacent to the nerve trunk, several endothelium-lined blood spaces were still patent, and some contained blood elements.

In a sagittal section through the hypophysis (fig. 2*A*) there was considerable distortion of the structural pattern of the gland. The major portion of the anterior lobe was replaced by a diffuse growth of tumor cells with displacement of the remaining glandular tissue to the dorsal region of the gland and to the region of the pars intermedia. The region of the pars intermedia consisted of many colloid-filled follicles, which almost completely separated the anterior and posterior lobes and extended posteriorly for some distance into the neural portion. The capsule of the gland was markedly thickened.



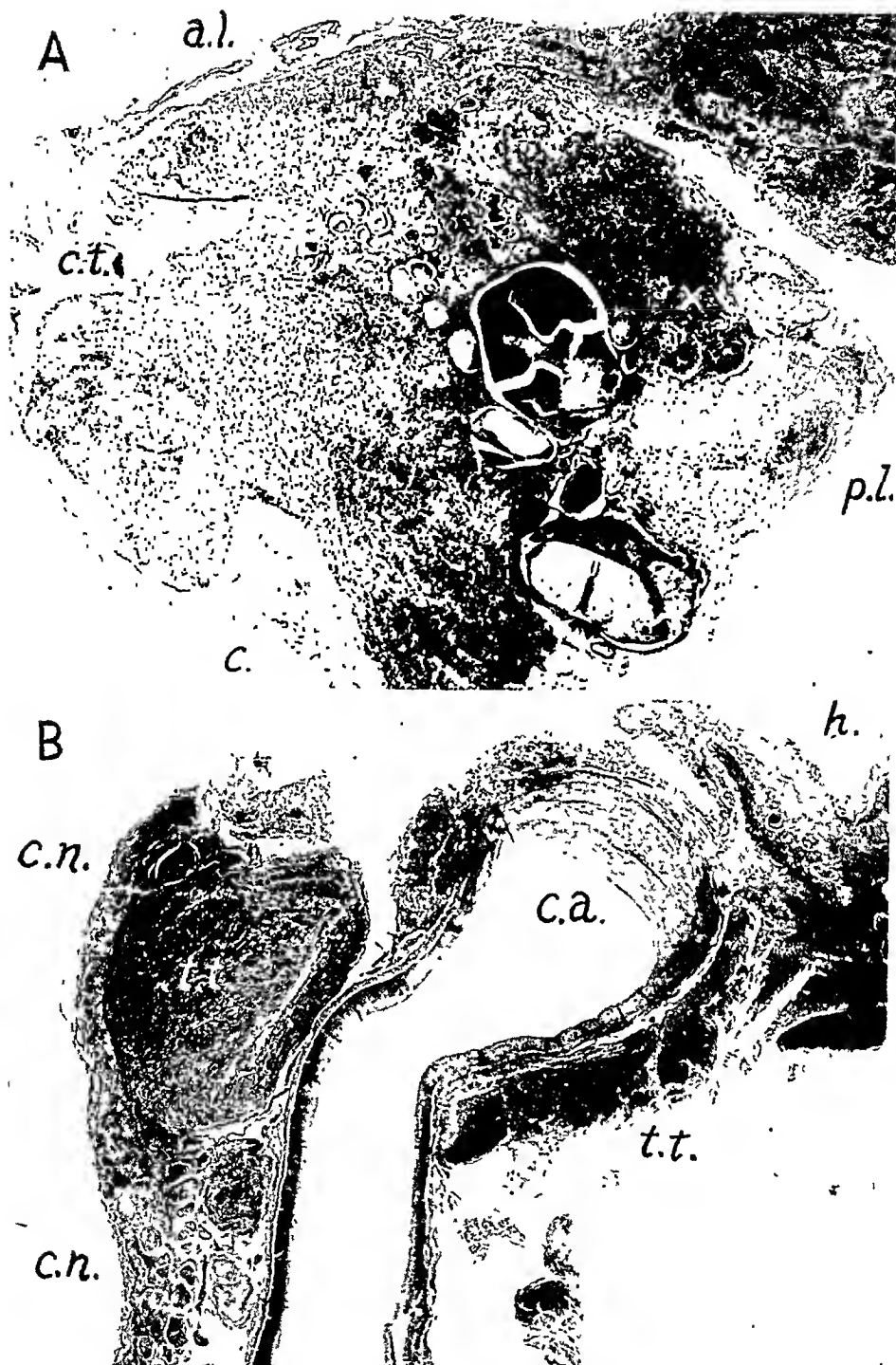


Fig. 2.—*A*, sagittal section of the hypophysis through the infundibular stalk ( $\times 0.18$ ); *p.l.*, posterior lobe; *a.l.*, tongue of anterior lobe tissue; *t.t.*, tumor tissue; *c.t.*, connective tissue wall separating the tumor area from the remaining anterior lobe; *c.*, thickened capsule. Note the numerous colloid-filled follicles and cysts in the region of the pars intermedia and extending into the posterior lobe at *X*, below which is a dense connective tissue scar. *B*, coronal section through a cavernous sinus and the lateral portion of the hypophysis (approximately  $\times 6.7$ ): *h.*, lateral portion of the hypophysis, in the region of the pars intermedia; *c.a.*, carotid artery, showing atherosclerotic changes; *c.n.*, trunks of the third, fourth, fifth and sixth cranial nerves; *t.t.*, tumor tissue.

Each element will be discussed separately in detail. Cytologically, the growth within the pituitary gland and the larger masses in the cavernous sinuses showed only a few minor differences (fig. 3).

The cells varied in size from two to four times the diameter of a red blood cell. They varied considerably in shape, but the majority were round to polygonal. The cytoplasm was coarsely granular, and many cells had numerous small vacuoles. The granules stained light pink with eosin, but only rare cells within the cavernous sinuses stained with orange G. However, orange G-staining granules were

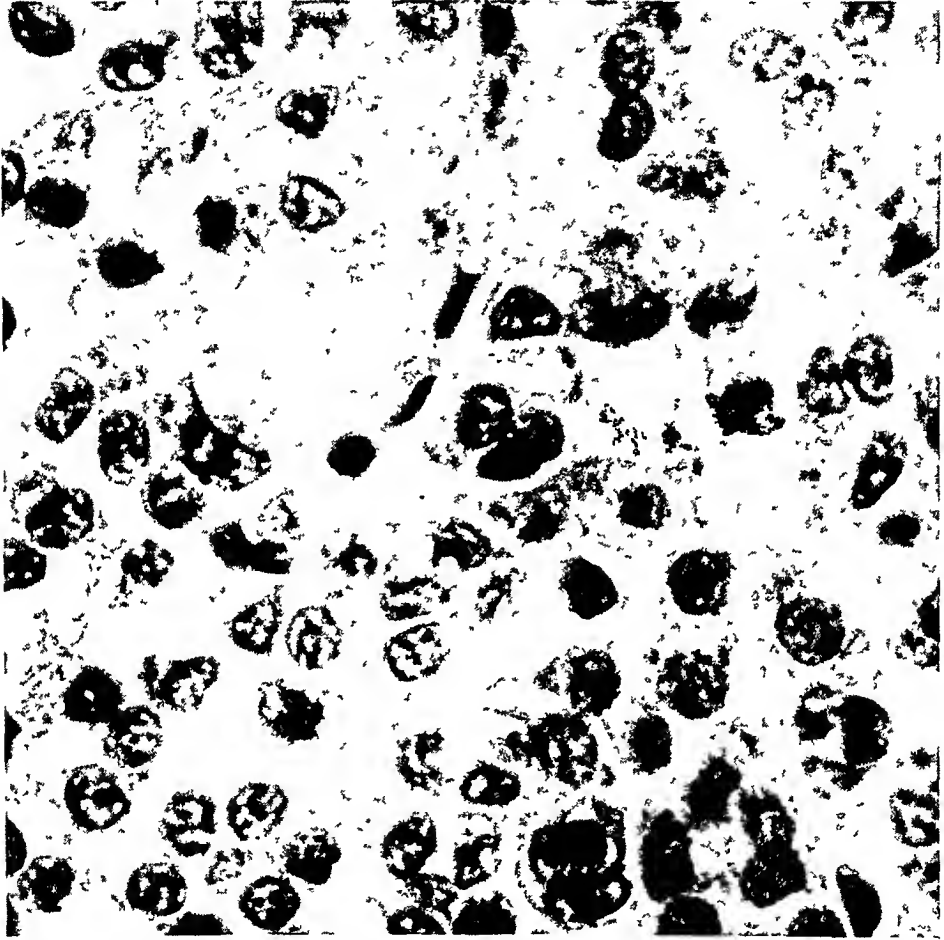


Fig. 3.—Tumor area in the anterior lobe ( $\times 800$ ). Note occasional binucleated cells.

more numerous but still infrequent in the tumor cells within the anterior lobe. Many cells showed an accumulation of hyaline droplets in the cytoplasm, and in some almost all the cytoplasm was replaced by this homogeneous material. This hyaline change has been described by Bailey and Cushing.<sup>4</sup>

The nuclei varied in size, and frequently a nucleus occupied the major portion of the cell. Binucleated and multinucleated cells were numerous, and most of them were very large. The frequency of this finding has been noted by Bailey

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4. Bailey, P., and Cushing, C.: *Am. J. Path.* 4:545, 1928

and Davidoff.<sup>5</sup> Chromatic substance was abundant, but large vesicular nuclei were also present. Many cells had small pyknotic nuclei. Mitotic figures were not observed.

The cell growth was on the whole diffuse with a very fine fibrillar connective tissue framework and few capillaries. In several areas there was a more abundant loose connective tissue stroma. In the region of the pars intermedia anterior to the colloid-distended follicles there was an insensible merging of the tumor growth with the remaining glandular tissue of the anterior lobe. However, superiorly, anteriorly and inferiorly the tumor was well demarcated by a heavy overgrowth of collagenous connective tissue.

In the cavernous sinus, there was a tendency to papillary growth along connective tissue stalks, and a few colloid-filled follicles were also noted. Some of the giant cells had large, irregularly shaped vesicular nuclei and large nucleoli.

In the anterior lobe the remaining glandular tissue was confined to a thin layer of cells anterior to the cysts of the region of the pars intermedia, to masses of cells in between these cysts and to a larger triangular mass of tissue in the dorsal and posterior region, in front of the attachment of the infundibular stalk (fig. 2 A).

The cells in front of the pars intermedia were large "chromophobe" cells with large vesicular nuclei and hydropic granular cytoplasm. There was both a diffuse and a follicular growth. A few ripe basophils and rare acidophils were present in this area. At one level in this region transitional basophils were numerous.

In between and to a lesser extent in front of the many colloid-filled cysts of the pars intermedia were large masses of ripe basophils. The largest of these nodules was one half the diameter of a low power field.

The tongue of anterior lobe tissue in the dorsal region of the gland consisted almost entirely of columns and follicles of large pleomorphic transitional basophil cells. Colloid masses, some of which were calcified, were present in several follicles. The cells were unusually large, with light blue finely granular cytoplasm and large vesicular nuclei with prominent nucleoli. Ripe basophils were present in this area in small numbers. A few small ripe acidophils and rare large transitional acidophils were also present in this zone.

In the posterior lobe the nerve tissue appeared more compact than it normally is. Pigmented cells were numerous, and there was only a very slight basophilic infiltration from the pars intermedia. Near the inferior surface there was a large area of replacement of the nerve tissue by collagenous tissue. Beneath this scarred area the pituicytes were numerous and arranged in anastomosing columns.

The capsule over the entire gland was greatly thickened and contained small deposits of osteoid tissue.

The infundibular stalk was thick and contained numerous pigmented cells. Small transitional basophils were present on its superior surface.

*Anatomic Diagnosis.*—The conditions diagnosed were: malignant adenoma of the anterior lobe of the hypophysis, with infiltration of both cavernous sinuses; enlargement of the liver, spleen, kidneys, pancreas and heart; hypertrophy of the adrenal cortex; colloid goiter; hypertrophy of the parathyroid glands; atrophy of the seminiferous tubules of the testes; multiple cutaneous verrucae; generalized arteriosclerosis; sclerosis of the coronary arteries; recent and healed microscopic infarcts

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5. Bailey, P., and Davidoff, L. M.: *Am. J. Path.* 1:185, 1925.

of the myocardium; bilateral hydrothorax; chronic tuberculosis of the left lung with cavitation; tuberculosis of the prostate, left epididymis, seminal vesicles and vas deferens; adenocarcinoma of the colon.

#### COMMENT

This case presents several unusual clinical and pathologic findings, which will be discussed under several heads.

*Sella Turcica.*—There was no roentgenographic evidence of enlargement of the sella turcica over a period of twenty-four years. Postmortem examination revealed a pituitary gland of normal size with replacement of the major portion of the anterior lobe by tumor cells and almost complete filling of the blood spaces of both cavernous sinuses by these elements.

As regards the presence or absence of sellar change in acromegaly or other pituitary disorders, it must be emphasized that the pituitary gland can double or even treble its weight without encroaching on its bony envelop. This is accomplished by obliteration of the perihypophysial blood sinus, by lateral growth in the direction of the cavernous sinuses and in upward expansion. Rarely, there may be a tumor in the sphenoid sinus arising from remnants of the hypophysial duct (Erdheim<sup>6</sup>), and in such cases there may be no sellar changes. So, except in cases with marked enlargement of the hypophysis, the sella need show no enlargement.

The normal variations of the sella also must be taken into consideration. Howe<sup>7</sup> stated that the pituitary fossa is normally subject to great variations in size and outline and gives no information concerning the condition of the pituitary gland unless there is marked sellar enlargement or absorption of some portion of the bony envelop. This has also been our observation in many hundreds of autopsies. However, in 93 of 100 cases of acromegaly reviewed by Davidoff<sup>8</sup> there was definite roentgenographic evidence of enlargement of the sella turcica, and in all but 8 of 248 cases reviewed by Atkinson<sup>2a</sup> the hypophysis was enlarged. Therefore, from both clinical and pathologic studies in instances of clinical acromegaly it appears that the pituitary tumor has reached a sufficient size in over 90 per cent of all cases to produce changes in the sella turcica.

*Size of the Pituitary Gland.*—In the older literature there are reports of a number of cases of acromegaly with a pituitary gland of normal size. Petrén<sup>9</sup> cited 6 cases and reported 1 of his own. Petrén's case

6. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **46**:233, 1909.

7. Howe, H. S.: Neurol. Bull. **2**:233, 1919.

8. Davidoff, L. M.: Endocrinology **10**:461, 1926.

9. Petrén, K.: Virchows Arch. f. path. Anat. **190**:1, 1907.

was that of a man of 50 years with concomitant syringomyelia. Petré<sup>n</sup> stated that the hypophysis was not enlarged and showed no microscopic changes. Bailey and Davidoff<sup>5</sup> doubted that Petré<sup>n</sup>'s case was one of true acromegaly. But even granting the authenticity of the case it is important to note that the gland weighed 800 mg., which is definitely above the average but within the limits of normal for males (Rasmussen<sup>10</sup>). In an experience covering many hundreds of pituitary glands, weighed and carefully examined microscopically, we have never seen a gland weighing as much as 800 mg. that failed to show some histologic changes, either hyperplasia, adenoma formation, tumor metastasis, hemorrhage or inflammation. Israel<sup>11</sup> reported another case of acromegaly with a "normal"-sized gland. The patient was a 54 year old man. The hypophysis weighed 720 mg., which is also slightly above the average. Israel further stated that a pituitary gland weighing as much as 1,000 mg. is not to be considered anomalous in view of the larger size of the body. This view is contrary to Rasmussen's<sup>10</sup> quantitative studies, which revealed only a moderate correlation between stature and the anterior lobe. Rasmussen could attempt no correlation between body weight and the anterior lobe because the weight at death was rarely the true weight.

The case of Lewis<sup>12</sup> appears to be one of early and relatively mild acromegaly. The hypophysis was not weighed or measured but appeared normal grossly. Microscopically, there was hyperplasia of the acidophilic cells with reduction in the number of chromophobes and basophils. There were no mitoses and no localized tumor.

It is thus clear that the many reports of "normal" size of the pituitary gland in the presence of acromegaly must be considered with reservations. While it is quite possible that the pituitary gland may be only slightly enlarged in cases of acromegaly, it is doubtful whether a careful cytologic study would not reveal evidences at least of hyperplasia in every instance.

In contrast to many of the ambiguous reports on acromegaly is that of Erdheim.<sup>13</sup> His patient was a 43 year old man with dementia paralytica and acromegaly. The hypophysis weighed only 1 Gm., and the sella was not enlarged. There was an oval tumor, 11.6 mm. in diameter, in the anterior lobe. Microscopically, it was acidophilic adenoma. This case is similar to case 1 of Cushing and Davidoff,<sup>2a</sup> an instance of acromegaly of thirty years' duration in a man. The sella

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10. Rasmussen, A. T.: *A. Research Nerv. & Ment. Dis., Proc.* (1936) **17**: 118, 1938.

11. Israel, O.: *Virchows Arch. f. path. Anat.* **164**:344, 1901.

12. Lewis, D. D.: *Bull. Johns Hopkins Hosp.* **16**:157, 1905.

13. Erdheim, J.: *Frankfurt. Ztschr. f. Path.* **4**:70, 1910.

turcica was not enlarged, and the pituitary gland was of normal size. A tumor diagnosed as acidophilic adenoma was present within the anterior lobe.

It must be emphasized that the absolute weight of the gland at death gives no accurate index of the size of the gland during life. The pituitary gland is subject to marked and rapid changes in size and weight, depending on a number of agents which act on it. In pregnancy in a multipara, for example (Erdheim and Stumme<sup>14</sup>), the gland may weigh as much as 1,650 mg. at term and within a relatively short period after delivery return almost to its previous size. In instances of acromegaly one must consider the stage of the disease. One would expect the gland to weigh considerably less in the burned-out stage than during the period of marked clinical activity. Many of the glands of "normal" size reported in the older literature may have been larger at some time preceding the death of the patient. In our case the gland was not weighed because it was left attached to the tumor masses in the cavernous sinuses, but its dimensions were well within normal limits. The total mass of the tumor growth, as can be judged from the photograph (fig. 1 B), was many times that of a normal gland.

*Nerve Involvement and the Cavernous Sinuses.*—Symptoms and signs referable to the visual apparatus occur in a large proportion of cases of acromegaly. In practically all instances these are due largely to upward, and to a much lesser extent to lateral, expansion of the pituitary tumor. The onset and course of the visual symptoms vary with the rapidity and direction of the tumor growth and with the secondary changes, such as hemorrhage or cyst formation.

In the case under discussion there were no visual symptoms until three days after the operation, at which time there developed left homonymous hemianopia. This and the other visual manifestations must have been due to the operative trauma, for they completely disappeared within a period of one month after the operation. Numerous careful neurologic examinations revealed only minimal signs, even twenty years later. This is all the more remarkable in view of what was found at the postmortem examination.

Weinberger, Adler and Grant<sup>15</sup> discussed the problem of nerve involvement in pituitary adenoma with especial reference to the ocular nerves and their implication through infiltration of the cavernous sinuses by tumor cells. Infiltration of one or both cavernous sinuses by tumor cells may result when an invasive neoplasm infiltrates the region of the

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14. Erdheim, J., and Stumme, E.: Beitr. z. path. Anat. u. z. allg. Path. **46**: 1, 1909.

15. Weinberger, L. M.; Adler, F. H., and Grant, F. C.: Arch. Ophth. **24**: 1197, 1940.

sella turcica from any direction. Erdheim<sup>16</sup> described a case of complete filling of the cavernous sinuses and destruction of the surrounding bony structure by a pituitary cancer.

However, there is clinical evidence to assume that in this case the infiltration of the cavernous sinuses occurred early in the course of the disease and persisted over a period of many years. Furthermore, there were relatively few signs that pointed to this phenomenon. One must reason in retrospect in order to establish the sequence of events.

The illness began with headaches. These were probably due to stretching of the capsule of the pituitary gland by the tumor or to distortion and irritation of the carotid arteries (Ray and Wolff<sup>17</sup>). After two years there was definite radiation of the pains to the face. The facial pains were severe and described as "pins and needles." These facial pains were due to irritation of the first two divisions of the trigeminal nerve.

In November 1915 the only neurologic sign was a narrow left palpebral fissure. This was due to compression of the left oculomotor nerve. However, a month later a competent observer noticed that the veins of the upper eyelids were dilated. Following the craniotomy, the cranial nerve signs and symptoms appeared, and these are detailed in the clinical history. However, these phenomena disappeared one month after the operation, and even the facial pains almost completely subsided. Nineteen years after the operation, the neurologic signs were still minimal.

It would seem that early in the course of the disease the cavernous sinuses become infiltrated by tumor cells from the anterior lobe. This may have occurred by invasion through the venous channels which empty into the cavernous sinuses or by direct invasion through the capsule of the gland. Schaeffer<sup>18</sup> stressed the importance of the diaphragm of the sella in influencing the direction of growth of a pituitary tumor. A strong and complete diaphragm would prevent upward expansion in the direction of the optic chiasm and would direct growth in the direction of less resistance, i. e., laterally.

The pains in the face, the partial ptosis of the left eyelid and the temporary dilatation of the veins of the upper eyelids indicated some involvement of the cavernous sinuses. The process must have been a gradual one, since the classic picture of thrombosis of the cavernous sinus was never evident. There was ample time for the establishment of anastomotic channels to care for the drainage of these sinuses. The appearance of frank neurologic signs several days after the operation was very likely due to operative trauma.

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16. Erdheim, J.: *Ergebn. d. allg. Path. u. path. Anat.* **21**:482, 1926.

17. Ray, B. S., and Wolff, H. G.: *Arch. Surg.* **41**:813, 1940.

18. Schaeffer, J. P.: *Anat. Rec.* **28**:243, 1924.

The cavernous sinuses have connections in several directions (Walsh<sup>19</sup>). Through numerous venous channels there are communications between the cavernous sinuses and the veins of the scalp, calvarium, orbit, face, cerebral hemispheres and internal jugular veins. Furthermore, Atkinson<sup>2a</sup> pointed out that in acromegaly the venous sinuses and emissary veins are enlarged. This would aid in the establishment of a collateral circulation.

In a series of 14 cases of pituitary adenoma with clinical signs of infiltration of the cavernous sinus Weinberger, Adler and Grant<sup>15</sup> found proptosis in only 3 instances. With infiltration of a cavernous sinus by tumor cells, which is a relatively slow process, there is adequate time for the establishment of a collateral venous circulation. Hence, the vascular phenomena of cavernous sinus thrombosis are infrequently seen in these cases. Furthermore, Eagleton<sup>20</sup> pointed out that chemosis and swelling of the lids in cavernous sinus thrombophlebitis are seldom due to venous obstruction alone. Suppuration, with its lymphedema, must be added to produce severe chemosis.

In instances of recovery from acute septic thrombosis of the cavernous sinuses (Schall<sup>21</sup>) subsidence of the engorgement of the orbital vessels requires several weeks to several months.

A careful search of the literature has failed to reveal a similar case of such long-standing infiltration of both cavernous sinuses by tumor cells. Bergstrand<sup>22</sup> reported a case of Cushing's syndrome in a 42 year old woman with a basophilic adenoma of the anterior lobe which broke through the right side of the gland into the cavernous sinus and grew around the carotid artery and into the lateral wall of the sinus, so that the third and fourth cranial nerves and in places the ophthalmic nerve were surrounded by tumor tissue. The fundi were negative. There was bilateral chemosis of the eyeballs. However, this was a late phenomenon.

One of the cases reported by Weinberger, Adler and Grant<sup>15</sup> (case 7) was that of a 48 year old acromegalic man. There was complete paralysis of the muscles of the right eye with ptosis and a dilated fixed pupil. Bitemporal hemianopia was present. Vision was 1/60 in the right eye and 6/60 in the left. The sella turcica was expanded and eroded, and both sphenoidal fissures were eroded. A tumor, diagnosed as acidophilic adenoma, was removed at operation. Six years after operation there was residual impairment of upward and downward

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19. Walsh, F. B.: *Arch. Ophth.* **17**:46, 1937.

20. Eagleton, W. P.: *Cavernous Sinus Thrombophlebitis and Allied Septic and Traumatic Lesions of the Basal Venous Sinuses: A Clinical Study of Blood Stream Infection*, New York, The Macmillan Company, 1926.

21. Schall, L. A.: *J. A. M. A.* **117**:581, 1941.

22. Bergstrand, H.: *Virchows Arch. f. path. Anat.* **293**:413, 1934.



rotation of the globe and partial ptosis. The authors stated that the cavernous sinus was encroached on by the tumor even though this could not be demonstrated at the operating table. However, in this case the tumor in the sella had reached a large size by the time the cavernous sinuses were implicated.

Erdheim<sup>23</sup> reported a case of acromegaly of twenty-two years' duration in a 48 year old woman who showed involvement of the cavernous sinuses and optic chiasm late in the course of the disease. There were bitemporal hemianopia with diminution of the visual fields, beginning atrophy of the right optic nerve and marked atrophy of the left optic nerve. The left pupil was wide, with a poor reaction to light, and the eyeball was turned outward. The patient died two days postoperatively. At autopsy the sella was greatly enlarged. The pituitary tumor grew upward and compressed the optic chiasm and the third ventricle. Both cavernous sinuses were filled with tumor. Microscopically, the tumor showed loss of acidophilic granules. In this case also, the involvement of the cavernous sinuses was a late phenomenon, and the pituitary tumor was large.

*Cytologic Character of Acromegaly.*—From the standpoint of cytology the pituitary adenoma in acromegaly varies with the stage of the disease and the degree of clinical activity (Bailey and Cushing<sup>4</sup>). In practically every case there occurs some hyperplasia or adenoma formation of acidophilic cells at some time during the evolution of the disease. Bailey and Davidoff,<sup>5</sup> Bailey and Cushing<sup>4</sup> and Cushing and Davidoff<sup>24</sup> have fully discussed this subject and have presented a full review of the literature.

Fränkel, Stadelman and Benda<sup>24</sup> were the first to study carefully the changes in the hypophysis in a series of cases of acromegaly. They recognized that hyperplasia of the chromophil cells, probably indicating an excessive activity of the gland, was the important factor in the production of the disease. The importance of understanding the life cycle of the acidophil cell is clear from the report of Cagnetto,<sup>25</sup> who in 1907 cast some doubt on the hypophysial origin of the disease on the basis of a case of acromegaly with a pituitary tumor consisting entirely of chromophobe cells. Other similar cases have been reported. But Cushing and Davidoff<sup>24</sup> pointed out in their monograph that the appearance of the acidophil cells may vary from the fully granular forms to those with fine dustlike granules. The granules may even disappear in the end stages of the disease, when there occurs a state of pituitary

23. Erdheim, J.: Virchows Arch. f. path. Anat. **281**:197, 1931.

24. Fränkel, A.; Stadelman, E., and Benda, C.: Deutsche med. Wchnschr. **27**:513, 536 and 564, 1901.

25. Cagnetto, G.: Virchows Arch. f. path. Anat. **187**:197, 1907.

insufficiency. Krumbhaar<sup>26</sup> had pointed out earlier that in instances of acromegaly with nonacidophilic tumor of the pituitary gland the possibility of progression beyond the acidophilic stage must be considered. Severinghaus<sup>3</sup> also emphasized the point that adenoma of apparent chromophobe cells could be associated with symptoms attributed to hypersecretion of a chromophil type. This is due to a discharge of secretion so rapid that no accumulation of granules occurs.

Severinghaus<sup>3</sup> gave a complete survey of the subject of cell interrelationships in the anterior lobe of the pituitary gland. The dynamic concept that he presented helps to elucidate many apparently contradictory features of pituitary cytology. The markedly labile reaction of the anterior lobe of the pituitary gland of the rabbit in response to thyroidectomy and thyroid feeding has been demonstrated by Marine, Rosen and Spark.<sup>27</sup> The profound changes in the anterior lobe of the pituitary gland produced in several species of animals by the administration of pregnancy urine extracts or estrogens have been demonstrated by many investigators.<sup>28</sup>

The entire question of hyperplasia and adenoma formation in the anterior lobe must therefore be reconsidered in the light of this experimental work. Huge tumors of the anterior lobe have recently been produced in castrated and normal rats of both sexes by the administration of diethylstilbestrol (Nelson<sup>29</sup>) and in castrated and partially adrenalectomized male rats by the injection of estradiol dipropionate (Rosen<sup>30</sup>).

Careful histologic study of the pituitary gland revealed that approximately three fourths of the glandular tissue of the anterior lobe was replaced by a growth of tumor cells. These tumor cells were obviously of limited growth potentiality, for over a period of many years they failed to break through the cysts of the pars intermedia into the posterior lobe or through the diaphragm of the sella to implicate the optic chiasm. Nor was there any invasion through the thick layer of connective tissue which separated the tumor tissue from the large tongue of hypertrophied basophils in the dorsal region of the anterior lobe (fig. 2 A).

Morphologically, there was no infiltration of the perineural sheaths of any of the nerves that traverse the cavernous sinuses or their walls, but there was infiltration of the walls of the internal carotid arteries.

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26. Krumbhaar, E. B.: *M. Clin. North America* 5:927, 1921.

27. Marine, D.; Rosen, S. H., and Spark, C.: *Proc. Soc. Exper. Biol. & Med.* 32:803, 1935.

28. Severinghaus, A. E.: *Anat. Rec.* 60:43, 1934. Cramer, W., and Horning, E. S.: *Lancet* 1:1056, 1936. Weil, A., and Zondek, B.: *Endocrinology* 25:114, 1939.

29. Nelson, W. O.: *Am. J. Physiol.* 133:398, 1941.

30. Rosen, S. H.: Unpublished data.

There was no destruction of bone, and only microscopic infiltration of dura. Hence, on the basis of the heterotopia of the growth and the atypical appearance of the cells, one must make the diagnosis of malignant adenoma, in spite of the limited growth potential of long standing.

*Cytologic Character of the Remaining Anterior Lobe.*—The cytologic observations on the remaining glandular tissue are of great interest. Few ripe acidophils were observed. A moderate number of large hydropic "chromophobe" cells were noted anterior to the region of the pars intermedia. The majority of the glandular cells consisted of ripe and transitional basophil cells. The ripe basophils were grouped in small adenomatous nodules in the pars intermedia zone, while most of the transitional basophils were located in the dorsal region of the gland.

These transitional basophils were of striking appearance. They were two to three times the size of normal ripe basophils and had large vesicular nuclei with prominent nucleoli. There was marked pleomorphism.

In a correlated study of several hundreds of pituitary glands we have observed that degranulation of basophil cells is frequently coincidental with the finding of diminished spermatogenesis. Experimentally, degranulation of the basophil cells can be brought about in several species of animals by administration of an estrogen and by thyroidectomy. Loss of basophilic granules also occurs in pregnancy. In the case under discussion there was not only fairly marked diminution of spermatogenesis but extensive and long-standing replacement of anterior lobe tissue by tumor cells. The hypertrophy of the basophil cells may thus also represent an attempt at regeneration of active secreting glandular elements. This is analogous to the hyperplasia of thyroid remnants after subtotal thyroidectomy (Halsted <sup>31</sup>; Marine and Lenhart <sup>32</sup>).

*Other Features.*—The remaining features of this case, namely, the occurrence of diabetes mellitus early in the course of the disease, the presence of marked enlargement of the thyroid gland and the changes in the gonadal sphere are too well known to require comment. These related hormonal disturbances are readily understood in the light of the newer information regarding the function of the hypophysis and its dominant role in the regulation of the endocrine system.

#### SUMMARY

This instance of acromegaly was observed clinically over a period of twenty-one years. The period of active growth was relatively short. This may have been due to early exhaustion of the acidophilic cells, as

31. Halsted, W. S.: Johns Hopkins Hosp. Rep. 1:373, 1896.

32. Marine, D., and Lenhart, C. H.: Bull. Johns Hopkins Hosp. 20:131, 1909.

evidenced by loss of granules. The most disabling symptoms were severe headaches, later those of diabetes, then those of tuberculosis. Death was due to sclerosis of the coronary arteries with acute terminal insufficiency of these arteries.

The headaches were severe enough to warrant surgical exploration of the pituitary gland.

The diabetes occurred early in the course of the disease and showed marked spontaneous fluctuations in severity. The thyroid enlargement also was observed at the beginning of the disease. Both of these phenomena, together with the gonadal dysfunction, are easily understood in the light of present knowledge of the trophic functions of the anterior lobe of the pituitary gland.

The hypophysis showed no enlargement. The main bulk of the tumor proliferated within the cavernous sinuses. The remaining pituitary tissue underwent hypertrophy. Infiltration of the cavernous sinuses was never suspected clinically, but must have occurred early in the course of the disease and persisted in a more or less static condition until death.

At the time of death few tumor cells contained acidophilic granules. In fact, even in the remaining pituitary tissue acidophils were sparse. Yet, this finding is not incompatible with the theory that acromegaly is due to hyperfunction of the acidophilic cells. This hyperfunction is evidenced in over 95 per cent of cases of acromegaly by some hyperplasia or adenoma formation of these cells. In about 20 per cent of the cases the tumor becomes cancerous. In this case, the cells not only lost their granules but also invaded the cavernous sinuses, the dura and the outer layers of the internal carotid arteries.

# Case Reports

## METASTATIC CARCINOMA OF SKELETAL MUSCLES

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Few reports of multiple metastases of carcinoma to skeletal muscles are found in the literature. The reasons for the rarity of metastatic tumors in skeletal muscles are not well understood. The present report is published because of the infrequent occurrence of metastatic carcinoma in several muscles of the body.

It was stated by Willis<sup>1</sup> that "true blood-borne metastases in skeletal muscles are exceedingly rare." He reviewed 15 cases reported in the literature and presented 4 additional ones. In 9 of these 19 cases there were multiple metastases in muscles. The muscles most frequently involved were those of the abdominal wall, the pectoral muscles, the deltoid, psoas and thigh muscles. The primary tumors in the 19 cases were given as follows: 6 were ocular tumors or melanotic, 2 were renal, 2 were pulmonary, 2 were thyroidal and 6 were located in an external ear, the pharynx, the liver, the duodenum, the tail of the pancreas, the foot (sarcoma) and the face (glioma), respectively. In only 2 instances were the metastases present in several muscles. In the case reported by Bauer<sup>2</sup> the primary tumor was in the kidney, and in case 242 of Willis<sup>1</sup> it was in the pharynx and many muscles were affected. In 7 others, two to five muscle metastases were present.

In reviewing the literature on cancer of the pancreas it was found that Grauer,<sup>3</sup> in a table of 34 cases of pancreatic cancer, showed that muscles were involved in 2 cases, but he made no comment as to their location. Baldwin,<sup>4</sup> in a review of 50 cases of cancer of the pancreas, found metastases to muscles in 4 instances, with one muscle affected in each case as follows: the abdominal wall, the pyloric wall, the wall of the intestine and a psoas muscle. Flexner<sup>5</sup> demonstrated a case of cancer of the pancreas with metastases to a psoas muscle. Marten and Meyer<sup>6</sup> reported a case of pancreatic cancer in a man 57 years old with metastases to the heart and a rectus abdominis muscle. Ritchie<sup>7</sup> in 16 reported cases of metastatic carcinoma of the myocardium found that 2 of the tumors were primary in the pancreas. He also showed in a table (case 30:193) metastases to voluntary muscle from a primary tumor of the skin diagnosed as reticuloendothelioma, but he did not state what muscles were involved. Reports of tumor metastases in the myocardium are not so rare and are not discussed at this time, although the patient did have quite an extensive metastatic growth in the right side of the myocardium.

1. Willis, R. A.: *The Spread of Tumors in the Human Body*, London, J. & A. Churchill, 1934.

2. Bauer, T.: *Beitr. z. path. Anat. u. z. allg. Path.* **50**:532, 1911.

3. Grauer, F. W.: *Arch. Int. Med.* **63**:884, 1939

4. Baldwin, F. W.: *Philadelphia M. J.* **6**:1195, 1900.

5. Flexner, A.: *Bull. Johns Hopkins Hosp.* **5**:16, 1894.

6. Marten, M. E., and Meyer, L. M.: *Am. J. Cancer* **27**:106, 1936.

7. Ritchie, G.: *Am. J. Path.* **17**:483, 1941.

## REPORT OF A CASE

J. C., aged 54, had enjoyed good health until he was "gassed" in the war in Europe in 1918. Since then he had been troubled with asthma. He worked for the railroad company for many years. There was no history of cancer in the family.

His last illness began about a year prior to examination, with backache. The use of plasters and heat gave some relief at that time. About six months later he had what was thought to be the "flu" and was in bed for two weeks. About four months prior to examination his backache became worse; he also had pains in the chest and arms. He went to the Veterans Hospital, at Des Moines, Ia., for a needed rest and further treatment. A few weeks later an apparently diffuse induration of the left pectoralis major muscle was noticed. Other muscles became similarly involved in the succeeding weeks in the following order: the left biceps, the right sternocleidomastoid, the right pectoral and the lower part of the right latissimus dorsi. Considerable bladder trouble also developed with frequency and dysuria. A tentative diagnosis of myositis ossificans was made, but biopsy of the left pectoral muscle showed the presence of carcinoma in the muscle. His condition was slowly getting worse and more muscles were being involved. After a three months' stay at the Veterans Hospital he returned home. He remained at home about a week, but because of his critical condition he was taken to Mercy Hospital, in Cedar Rapids, Iowa.

The chief complaints when he entered the hospital were those of severe cystitis and severe pain and cramps in many muscles. He was unable to retain any food in his stomach and soon vomited fluids also. His condition rapidly became worse and he died ten days later.

At the necropsy extensive carcinoma of the tail of the pancreas was found with extension into the retroperitoneal tissues of the left side. Other muscles involved were the left diaphragm posteriorly, the left psoas, the right and left pectoral muscles, the right lower latissimus dorsi, the left biceps, the right sternocleidomastoid, the wall of the cecum, the wall of the urinary bladder and the right side of the myocardium in the region of the ventriculoauricular junction.

A large part of the left biceps muscle was replaced by tumor tissue and the arm had become fixed in a semiflexed position. The involvement of the left psoas muscle had caused thrombosis of the femoral vein with marked congestion and edema of the left leg about six days before death. He had no control of the urinary bladder while in the hospital. The prostate was small and was not involved. Most of the anterior and upper part of the bladder wall was infiltrated by tumor cells as shown by the microscopic sections. The liver, lungs, kidneys and spleen were not involved, although the capsule of the spleen was adherent to the growth in the pancreas. The left adrenal was involved by extension, as well as the capsule of the left kidney.

*Microscopic Examination.*—The tumor of the pancreas was found on microscopic sections to consist of rather small, deeply stained epithelial cells. The nuclei of these cells were relatively large. The tumor cells occurred in large diffuse masses for the most part and in smaller masses showing a tendency toward alveolar formation. There was little connective tissue stroma in the pancreas, but there was considerable diffuse scarring of the growth in the muscles. The tumor cells in the skeletal muscles, the myocardium, the wall of the urinary bladder and the cecum were small, deeply stained epithelial cells similar to those in the pancreas. There was considerable chronic inflammation of the muscles in the region of the tumor tissue. Biopsy of the left pectoralis major muscle by the Army Medical Museum, Washington, D. C., was reported to show metastatic carcinoma.

## COMMENT

The infrequent discovery of metastatic carcinoma in the skeletal muscles and the single metastasis or small number of metastases found in any one case attest to the rarity of metastatic carcinoma of muscles. A metastasis in a skeletal muscle when present is usually suspected during life because of the pain and impaired use of the muscle involved. A few of the theories explaining the localization of cancer metastases are the following:

1. The circulatory anatomy. This is thought to explain the frequency of metastasis to the liver of abdominal tumors and to the lungs of tumors in other parts of the body.

2. The frequent movements of muscles. This is thought to prevent the development of metastases in them.

3. The metabolic or biologic properties of the tissues. The liver is thought to be susceptible because of its rich carbohydrate content and its poor oxygenation. The rarity of metastases in skeletal muscles is thought to depend in some way on the metabolism of the muscles, in which lactic acid, an important product of cancer cells, plays an important part.

4. The metabolic activity of embolic tumor cells. According to Crile,<sup>8</sup> cancer cells must have a high capacity for the storage of electric charges, and this must be especially high for them to develop in muscles, which themselves have a high rate of metabolism.

5. The vascularity of the organ. Highly vascular organs appear to be less often involved by metastatic growths but appear to be quite often the sites of origin of tumors with widespread metastases. The thyroid and kidneys have a rich blood supply but are not often the site of metastases, although tumors of these organs often have widespread metastatic growths. The kidney is often infarcted in cases of endocarditis, however.

The frequent widespread metastasis of cancer of such organs as the thyroid, the kidney, the prostate and the pancreas and of some forms of cancer of the breast and stomach may be due to the development of high electric charges of the tumor cells, as mentioned, to the large number of tumor emboli that may be liberated from vascular tumors of these organs or to the fact that tumor cells developing in highly vascular organs or tumors may be capable of growing in the capillaries of the blood stream wherever they may lodge as emboli. The widespread metastasis of cancerous melanoma may be due to the fact that the cell from which it develops is a less differentiated type and to the tendency of this neoplasm to spread along and involve blood vessels.

## SUMMARY

Metastasis of carcinoma to skeletal muscles is rare. A case of primary carcinoma of the pancreas with metastases to many skeletal muscles, the myocardium and the muscles of a few viscera is reported.

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## SIMULTANEOUS LEIOMYOSARCOMA OF THE UTERUS AND PAPILLARY CARCINOMA OF THE OVARY

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Multiple cancer in a patient was first described by Billroth, in 1869. Since that time there have been numerous reports on the subject and wide interest has been stimulated concerning the relationships of the various tumors to each other. Warren and Gates<sup>1</sup> published a comprehensive review of the entire literature in 1932. They collected 1,259 cases exclusive of multiple myeloma and a few cases in which the pathologic diagnosis was considered uncertain. The age groups were essentially the same as with single cancer. Their calculated incidence of cases of multiple cancer among all cases of cancerous disease was 1.9 per cent for the total and 3.9 per cent for the Americas. It was their opinion that the occurrence was too frequent to be explained by mere coincidence. Orr<sup>2</sup> in a limited series of cases from the English literature found an incidence of 1.09 per cent. He calculated that if the occurrence were purely coincidental the incidence should range from 1.3 to 2.7 per cent. In other nonselected groups<sup>3</sup> the incidence has been reported as ranging from 1.7 to 2.7 per cent. Barnes<sup>4</sup> reported a review of the literature on 28 cases of simultaneous carcinoma and sarcoma of the uterus and added 2 of his own. He expressed the belief that in this type of tumor combination there must be a definite relationship, since the incidence is out of proportion with the coincident expectancy. All types of tumors and almost all combinations have been reported.<sup>5</sup> For a complete list of the variations, combinations and numbers of multiple cancers reference should be made to the paper of Warren and Gates.<sup>1</sup> Prior to that time only a single incidence of the type of tumor combination recorded in this report had been described.<sup>2</sup> This was a case of a 57 year old white woman with sarcoma arising from fibromyoma of the uterus associated with carcinoma of the ovary. The latter tumor had metastasized to the peritoneum. In 1934 Scudder and Fein<sup>6</sup> published a brief review of the literature and reported a similar instance of their own. The patient, a 52 year old white woman, had a large ovarian cyst removed at operation which on microscopic examination was found to be cancerous. No metastases were found, and the uterus was small and atrophic at the time. Six months later she returned with a watery discharge

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and an enlarged uterus. Removal of the uterus revealed leiomyosarcoma arising from leiomyoma of the uterus. Shortly after this the patient was found to have metastases of the latter tumor in the vaginal wall. In their paper they refer to 2 previous instances but cite only Orr's case.<sup>2</sup> Their bibliography did not refer to another case report of similar tumors. I could find no further like cases recorded since that time.

#### REPORT OF A CASE

F. L., a 58 year old Negro woman, was admitted to the gynecologic service of the Vanderbilt University Hospital in May 1941. She was the mother of a child who is living and well. Her health had been good prior to March 1941. At this time vague discomfort in the lower abdominal region developed. One month later the discomfort became definitely painful and combined with a sense of weight and bearing down. These symptoms progressed and she lost about 5 pounds (2.3 Kg.) in weight. Her history was otherwise negative. She denied having had venereal disease.

She was well developed and well nourished, showing slight evidence of loss of weight. The temperature was 98.6 F.; the pulse rate, 90; the respiratory rate, 20. The blood pressure was 150 systolic and 80 diastolic. She complained of moderate pain in the lower part of the abdomen. The head, neck, heart and lungs were recorded as normal. The abdomen was distended, and a large firm nodular mass was palpable in the lower quadrants, extending from the umbilicus to the symphysis pubis. The mass was thought to be smooth and movable. On pelvic examination it obliterated the normal landmarks.

The urine had a specific gravity of 1.017; the reaction was acid; there was no albumin; the results of a microscopic examination were negative.

The red blood cell count was 4,200,000; the hemoglobin content was 10 Gm.; the white blood cell count was 7,450; the differential count and the smear were normal; the nonprotein nitrogen was 25 mg. per hundred cubic centimeters. The Wassermann reaction was 4 plus on two occasions.

*Course.*—Because of a slight cough and fever, a roentgenogram of the chest was made, which revealed an aneurysm of the thoracic aorta. There was no history of signs or symptoms of cardiac decompensation. It was advised that she be given digitalis prophylaxis, with doses of 2 cat units (about 3 grains [0.19 Gm.]) twice a day. Following this there was a gradual rise in blood pressure from 150 systolic and 80 diastolic to 190 systolic and 120 diastolic during the course of three days. Late on the third day the patient complained of sudden severe pain in the lower thoracic region, radiating into the abdomen and the lower part of the pelvis. Abruptly she became weak, cold and clammy; the pulse was rapid, and the blood pressure was 100 systolic and undetermined diastolic. Morphine and a 25 per cent solution of pyridine betacarboxylic acid diethylamide (coramine) were given. She died within thirty minutes after the onset of the pain.

*Clinical Diagnoses.*—Syphilitic aneurysm of the aorta with rupture and multiple leiomyoma of the uterus were diagnosed.

*Autopsy.*—Gross Observations: The body was well developed, and there were no gross abnormalities of the head, eyes, ears, nose, mouth, neck or thorax. The abdomen was protuberant, and a large nodular mass, comparable in size to a five months' pregnancy, was palpable in the lower portion. A small amount of clear fluid was present in the peritoneal cavity. There was a cyst in the upper mesentery, which contained approximately 500 cc. of thin brown fluid. A large nodular mass was present in the lower part of the peritoneal cavity, to which the omentum

and many loops of intestine were adherent. It completely obliterated the pelvic organs. The peritoneum in this region was studded with small white firm nodules, varying from 2 to 8 mm. in diameter. The mesenteric and retroperitoneal lymph nodes were enlarged and firm and on cut section contained white tissue. The pleural cavities contained no fluid or blood. The pericardial sac was not remarkable. The heart was moderately enlarged, and the ventricular walls were increased in thickness. The endocardium and valves were normal. A large saccular mass was noted in the posterior and superior mediastinum about the aorta. When opened, it yielded about 800 cc. of blood. The walls of the sac appeared to be composed of periaortic connective tissue. The aorta itself was tortuous and dilated in its descending portion and presented three separate enlargements. The first and largest marked the termination of the arch. It measured 4 cm. in diameter and on cut section was found to be a dissecting aneurysm filled with an old thrombus. Its walls were intact. The second enlargement, 5 cm. below the first, was smaller and involved only a small part of the wall. A small intact mural thrombus was present on the corresponding intimal surface. The third dilatation was at the lower portion of the thoracic aorta and projected 1.5 cm. posteriorly. At its apex was a defect from which a thrombus protruded. This apparently represented the point of rupture of the aneurysm. Cut section showed the entire dilatation filled with a thrombus.

The liver was enlarged and showed congestion. The spleen, pancreas, adrenals and kidneys were normal on gross examination. The ureters were slightly dilated.

The abdominal mass incorporated all the pelvic organs except the bladder. The mass itself was composed of several large rounded nodules. One of these, in the region of the right ovary, was cystic and of a blue color. It was filled with serous fluid, and papillary growth was present on one portion of its wall. The remaining nodules were solid, although in some areas they had a soft consistency. The rectum was embedded in the mass posteriorly and was surrounded by soft white amorphous tissue. On cut section there were three spherical nodules in the mass, two of which were circumscribed, white and firm. They appeared to be composed of whorls and sheets of tissue. The other nodule was soft and showed evidence of degeneration, which was not sharply demarcated. The uterus was very small, and its lumen was intact. The uterus and the aforementioned nodules were embedded in a mass of white tissue, parts of which were like that present about the rectum, other parts being firmer with a gritty consistency. Areas of degeneration were present throughout this tissue. The tubes and ovaries could not be identified (fig. 1). Examination of the brain revealed no gross abnormalities.

**Microscopic Observations:** The aortic wall showed increased vascularity of the media and adventitia with plasma and round cell infiltration about the vessels. There was some scarring of the media, and acute and chronic destruction was noted at several points. Where the rupture occurred the aneurysmal wall was thinned out from the mouth of the sac to the apex, where it was entirely absent, leaving the thrombus in communication with the exterior.

The liver showed numerous scattered areas of focal necrosis. These varied in size from those involving one to two liver cells to others including one fourth of a lobule. The necrosis was acute and associated with plasma cell infiltration plus a few lymphocytes. The triads also showed plasma cell infiltration. Focal necrotic areas with cells of the same types present were found in the adrenal glands. In the kidneys there were large areas of plasma and round cell infiltration, associated in some areas with necrosis. Some of these areas of focal necrosis were characteristic of miliary gumma.

Microscopically, the uterus was atrophic, and its mucosa was intact and showed no evidence of cancer. There were abnormal cells in some of the lymphatics of the walls of the body and cervix. They were epithelial in character with large dark nuclei and pink cytoplasm. In some areas they exhibited a tendency to form glands but mainly were in compact groups. Sections including the tissue just outside the uterus contained large masses of these cells, which not only formed glands and cystic areas but produced a papillary structure within these spaces. Mitotic figures were frequent. Where the glandular and papillary structure was



Fig. 1.—Photograph of the pelvic mass cut in the central anteroposterior and superior-inferior plane showing papillary cystadenoma (*A*), leiomyoma (*B*), tumor mass (*C*) and uterus (*D*).

present the cells appeared columnar, although pseudostratification was also apparent (fig. 2 *A*). Sections from the soft white amorphous tissue about the rectum presented a different appearance. They were mainly composed of extremely cellular connective tissue. The cells for the most part were spindle shaped, were large and contained more chromatin in their nuclei than usual. Many of the round cell type were also present, and their general arrangement was in sheets, whorls and columns. Mitotic figures were abundant (fig. 2 *B*). Some of the sections from these areas showed the

epithelial cells already described infiltrating directly into this neoplastic connective tissue. They were present in groups, glands, cystic areas and papillary form. Lymph nodes about the aorta were extensively invaded by the epithelial tumor.

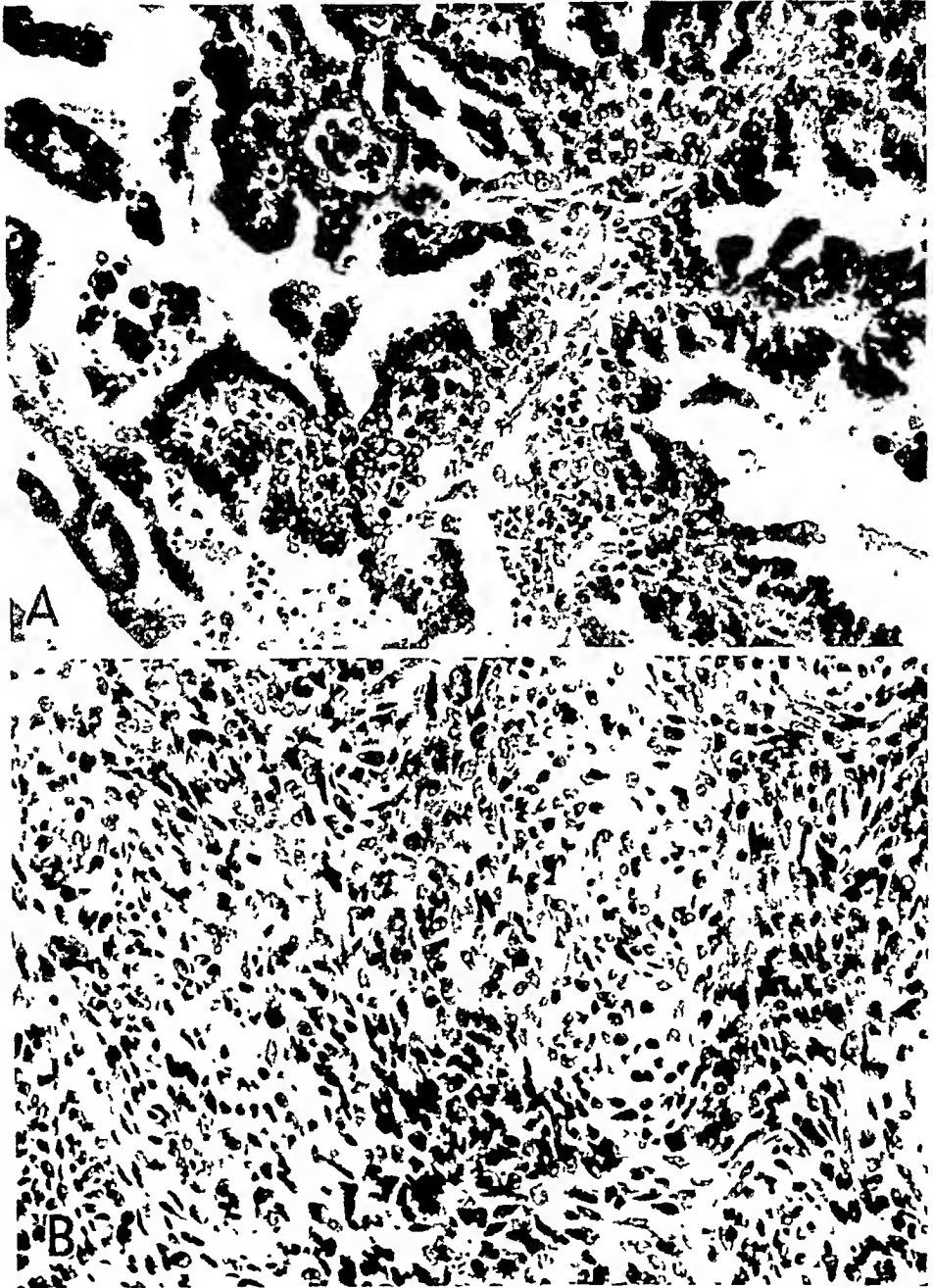


Fig. 2.—*A*, photomicrograph of papillary adenocarcinoma of the ovary in a peri-aortic lymph node; *B*, photomicrograph of leiomyosarcomatous tissue about the rectum.

The epithelial tumor described is characteristic of papillary adenocarcinoma of the ovary. It is present in the wall of the uterus, in isolated nodules, in the peritoneum and in the retroperitoneal lymph nodes. A large cyst is described

in the region of the right ovary, which showed microscopically the same type of cells in papillary form within the cyst. The uterine mucosa was found intact. This growth represents papillary adenocarcinoma of the ovary. The neoplastic connective tissue described is closely associated with the uterus and the multiple leiomyoma described. The cells have all the characteristics of leiomyosarcoma and have invaded the pelvis extensively. In some of the areas of metastasis the two tumors exist together. Mallory's aniline blue stain shows the typical appearance with only a part of the cells stained blue, the remainder taking the red stain. The diagnosis of leiomyosarcoma of the uterus cannot be questioned.

#### SUMMARY

A brief review of the literature on multiple cancer is given. The only 2 cases of simultaneous leiomyosarcoma of the uterus and carcinoma of the ovary found in the literature are summarized and the report of a third case is presented.

# General Reviews

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## EFFECTS OF RADIATION ON NORMAL TISSUES

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*(Continued from Page 931)*

### VIII. EFFECTS ON THE GONADS

#### THE TESTIS

Because of the sensitivity of the germinal epithelium to radiation and because of the great importance of this sensitivity, most of the experimental and clinical observations on the irradiated testis have been concerned with the spermatozoa and the cells from which they are derived. It must be remembered that the so-called reversible and irreversible changes (Ellinger), or temporary and permanent azoospermia, are not fundamentally different, varying only in the number of germinal epithelial cells destroyed.

The initial experiments of Albers-Schönberg demonstrated that a moderate amount of soft roentgen radiation (roentgen rays of long wave length and little penetrative power) given daily for periods of from fifteen to thirty minutes for a total of from one hundred and ninety-five to three hundred and seventy-seven minutes produced sterility in the 5 male rabbits and 6 male guinea pigs treated, without impairment of their sexual potency. On 8 of the 11 animals autopsies were made; 7 showed azoospermia and 1 oligonecrospermia. His observations were confirmed by Friebe, who made pathologic studies of the testes of these animals and found them to range from one third to one half the normal size and to show no spermatogenesis, but destruction of the epithelium lining the seminiferous tubules.

The first observations on man were reported by Philipp; these are concerned with a man of 25 and another of 31 years of age; both showed azoospermia several months after irradiation of the testes. One patient had been treated with soft rays in divided doses for one hundred and ninety-five minutes; the other, for three hundred and sixty-five minutes. In the case of the younger man, normal motile spermatozoa were still present at the end of the course of treatment, which required a period of thirty days. No examination of the testes themselves was made.

In 1905 Brown and Osgood reported 18 cases of azoospermia or oligonecrospermia in roentgen ray workers. Six of the group also

showed roentgen ray dermatitis of their hands. All of those examined, who had done extensive roentgen ray work for more than three years, showed complete azoospermia.

As a result of these early observations, adequate protection was provided in the great majority of instances of human exposure and the development of sterility became much rarer.

The first thorough experiments were made by Bergonié and Tribondeau, who irradiated the testes of the white rat while shielding the rest of the animal. The exposures to relatively soft roentgen rays varied in duration from two to ten minutes and were repeated at intervals ranging from one to eight days. In no animal was there damage to the overlying skin following treatment. On gross examination the testes were soft and very translucent as a result of edema, which was marked at the periphery but somewhat less in the central portions. The observations were controlled by the removal of the opposite testis just prior to the application of radiation. When the microscopic sections were examined, it was found that there were varying degrees of destruction of the germinal epithelium. Mitotic figures had disappeared. Large spermatocytes were rare, and those that persisted showed considerable fragmentation of the chromatin. The spermatids, the small spermatocytes and the spermatogonia persisted longer than did the large spermatocytes and showed nuclear pyknosis rather than fragmentation. Later, however, the pyknotic nuclei broke up, and their fragments usually disappeared, although survival of some bits of chromatin was not unusual. The spermatozoa were somewhat more resistant but gradually became adherent to one another and then dissolved. The Sertoli cells persisted and eventually proliferated to form a syncytial mass, which frequently filled the lumens of the tubules, apparently by amitotic division. The rapidity of destruction of the germinal epithelium was striking. All the testes removed a month and a half after the last series of treatments showed not only complete degeneration but removal of all traces of necrotic cells.

The destructive effect on germinal epithelium of radium as well was demonstrated by Thies, who exposed the testes of adult guinea pigs to 20 mg. of radium bromide for twenty-four hours. Fourteen days later spermatozoa were completely absent and the seminiferous epithelium was destroyed. The tubules were filled with masses of disintegrated tissue. The Sertoli cells were still in their normal situation, but their nuclei were somewhat swollen.

Variation of the sensitivity of the germinal epithelium in different species was pointed out by Regaud, who showed that cats were more difficult to sterilize than rodents. In heavily irradiated testes, germinal cells similar to those seen in immature mammals persist and after a time

divide, with later appearance of spermatogonia. As in rodents, the Sertoli cells are relatively resistant. However, the spermatogonia and the spermatocytes of the first order in the frog are sensitive, a single exposure of thirty minutes at relatively low voltage being sufficient to produce extensive nuclear degeneration (Amato).

A detailed consideration of cytologic changes due to radiation will be found in the observations of Barratt and Arnold on the testicles of the rat. These workers confirmed in general the observations made by others and added much detail of cytologic interest that is hardly pertinent to the present review. They emphasized the formation of fatty globules as a degenerative change in the cytoplasm of the spermatid. They also gave valuable time relations between the exposures and the various pathologic changes found. Necrosis has been observed in the spermatocytes of the first order and in spermatids as early as the third day following exposure to radiation. Vacuolation of the cytoplasm develops from the third to the seventh day, chiefly in the Sertoli cells. Multiple and abnormal mitoses occur in spermatocytes of the first order, appearing as early as twenty-four hours after the application of roentgen rays. Amitosis is marked in the Sertoli cells.

Spermatozoa isolated on being irradiated with radium (2 mg. or 7.4 mg.) (Hertwig) retain their power of penetration, penetrating the ovum, but lose their specific nuclear function; even slight exposure produces abnormalities in the offspring (Henshaw).

Tsuzuki showed that within twenty-four hours after 12 per cent of an erythema dose was delivered to the testis of the rabbit there was degeneration of the nuclei of spermatocytes; after seventy-two hours there was degeneration of spermatogonia, and after ninety-six hours a greater extent of the germinal epithelium was involved. More than 30 per cent of an erythema dose is required to give complete aspermia.

On the other hand, Pickhan, on the basis of irradiation of the gonads of *Drosophila*, expressed the belief that 20 r is a safe dose for the gonads.

Detailed mention of the interstitial cells is rare. Hu and Frazier reported no damage to the Leydig cells when rabbits' testes were given 4.4 erythema doses in six divided doses three days apart at 140 kilovolts. With this dose they found a few spermatogonia still present fifteen days after the last radiation, only the Sertoli cells and rare atypical spermatogonia at fifty-eight days and fibrosis at seventy-eight days.

In general it is assumed that the interstitial cells are undamaged by the usual therapeutic use of radiation (Ellinger) and that their apparent increase in number is due to atrophy of the tubules (Schinz and Slotopolsky).

The epididymis of irradiated testes is devoid of spermatozoa (Thies) and is usually shrunken.



## THE OVARY

Since changes induced in the ovary by radiation may be reflected by changes in the organ itself, by changes in the other genitalia and by changes in the embryo, it is necessary to consider these effects to some extent separately.

Atrophic changes in the ovaries themselves were first noted. Halberstaedter produced these changes in rabbits with relatively light, soft roentgen radiation. One ovary was irradiated; the opposite, shielded. The animals were killed ten days to three weeks afterward. The graafian follicles were smaller ten days after exposure and had almost completely disappeared by the fifteenth day. After the disappearance of the graafian follicles there remained clearly defined spaces with eosinophilic debris but without definite lining. There were no changes in the corpora lutea.

These results were confirmed by Krause and Ziegler, who found that the cells of the zona pellucida had degenerated after six hours of exposure to radiation from a "hard Mueller tube." In animals killed twenty-four hours later, the ova floated free in the liquor folliculi.

The quantitative studies of Bergonié and co-workers showed that an ovary irradiated for sixty minutes with the soft roentgen rays available in 1905 lost 42 per cent of its weight and that one irradiated for one hundred and forty minutes lost 85 per cent of its weight and was completely devoid of graafian follicles. These determinations were made one month after the use of radiation.

Saretzky discovered that the maturing or matured follicles were the most sensitive to radiation, and Okinchits produced follicular degeneration as well as shrinkage of stromal cells.

Although Simon had but few data (2 guinea pigs, 1 rabbit, 2 bitches and 1 human subject), his studies were careful. A guinea pig receiving 10 Kienböck units (1 erythema dose) was killed twelve hours after exposure and showed a hyaline residuum in place of the ova in the primary follicles. The ova in the more mature follicles showed varying degrees of degeneration. The remainder of the ovary was normal with the exception of scattered stellate hemorrhages. The second guinea pig received 12 Kienböck units (1.2 erythema doses) and was killed four months later. The follicular epithelium was almost entirely gone. The whole ovary was hyperemic. The stromal cells were swollen and glassy and had stained poorly. In the rabbit given 8 Kienböck units (0.8 erythema dose) changes similar to those in the guinea pig were observed at the end of a month, and at the end of one hundred and thirteen days the degenerative changes were marked. However, corpora lutea persisted. One bitch received 8 Kienböck units. Six days later an ovary was hyperemic with numerous hemorrhages in the stroma and the

follicles. Some ova had already disappeared, although there were a number of normal follicles. Three months later numerous corpora lutea were still present, but the graafian follicles were markedly degenerated. The second animal was given 16 Kienböck units (1.6 erythema dose), producing epilation and dermatitis. Three weeks after exposure to radiation the follicles showed marked degeneration and the ovary was hyperemic. Sixty-eight days later there had been extensive hemorrhage in the substance of the ovary and numerous follicles had become cystic. No normal follicles could be seen. The stromal cells were reduced in number, and their outlines were indistinct. In one human ovary, removed from a 42 year old woman after a total of 30 Kienböck units (3 erythema doses) had been administered, few follicles were present, and those showed degenerative changes similar to the changes in the lower animals. No evidence of regeneration was found in any of the ovaries examined.

As little as 50 to 100 r delivered to the bodies of white mice will produce degeneration of a portion of the ova in the ovary (Mahnert). After five weeks, shrinkage and degeneration of the stromal cells frequently occur. Specht, using moderately light doses of radiation in rabbits, noted some degenerative changes in the interstitial cells. This is contrary to the observations of Wintz (1927), who was able to destroy the germinal epithelium without apparent injury to the other ovarian tissues.

It has become clear, however, that it is difficult to establish any uniform sterilizing dose. Thus, while 0.2 to 0.1 human erythema dose produced skipping of one or two estrual periods in the rat, the only histologic change noted was ovarian congestion (Drips and Ford). With heavier doses of radiation, sufficient to produce sterilization, a few small follicles and primary oocytes remained uninjured even with the most intense radiation. Such animals, however, are not fertile. Young animals are somewhat more susceptible to injury than older ones (Fraenkel). The minimal sterilizing dose for the guinea pig is about 2,100 r, given in divided doses over six days (165 kilovolts peak; filter, 0.25 mm. copper + 1 mm. aluminum; focal skin distance, 50 cm.). It is difficult to produce an ovary in which the interstitial cells are still present and all ova have been destroyed, the so-called interstitial gland ovary (Genther).

Reifferscheid noted degeneration of the follicles and ova and occasional slight cortical bleeding in 6 human ovaries that had been irradiated.

In a 30 year old woman whose ovaries had been repeatedly irradiated over two and a half years, receiving a total of four castration doses, the follicles were gone, but not the interstitial tissue (Borak and Windholz). It has been stated that about twice as much radiation is required to destroy the ovary as to produce aspermatogenesis (Clark). If oogenesis

is active in the rabbit, it has been stated that 4,500 to 5,000 r are required to destroy the ovary (Gricoureff). Desjardins considered the ovaries as less sensitive than the white blood cells. It has been claimed that by ligating the ovarian blood vessels during a period in which the ovaries are irradiated the sensitivity of the ovary can be reduced (Ferroux and associates). Ford found that with 2,130 r the corpora lutea and the ovarian stroma of the gopher were unaffected in spite of massive destruction of the follicles. The corpora lutea are stated to be the most resistant portion of the ovary in the guinea pig (Fritschi) and other forms (Matthews; Rock and co-workers). Erhardt found that the luteal hormone is resistant to heavy doses of radiation. By irradiating the mouse with 200 to 500 r it is possible to cause disappearance of all ova in the larger follicles, but later recovery may be complete (Geller).

The effectiveness of small doses of roentgen rays may be increased out of proportion to the total energy. Thus Guthmann showed, on the basis of 291 cases, that 25 per cent of an erythema dose delivered to the human ovary was not proportionate in effect to 80 per cent of an erythema dose.

Cellular infiltration as a result of irradiation of the ovary is not marked, but some lymphoid infiltration may occur (Matthews).

While stimulation has been claimed to occur with small doses of roentgen rays (Moppett), this has not been proved.

The sterilizing dose in the rat with radium is 600 to 650 millicurie hours. Sixty-six per cent of radium-treated rats that lived for four months were sterile (Murphy). Murray, using the white mouse as an index, found that as little as 54 r produced some change in ovarian function; 150 r produced sterility in every instance. Schugt produced ovarian damage in mice with 54 r. Wintz (1928) stated that 34 per cent of an erythema dose completely and permanently stops ovulation and internal secretion in the human being and that 28 per cent produces temporary cessation only. Zondek expressed the belief that irradiation destroys maturing ova and does not permit the production of new ova.

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## IX. EFFECTS ON THE NERVOUS SYSTEM

## THE NERVES

The material dealing with radiation changes in nerves is contradictory and incomplete, and not easily evaluated. Some workers (e. g., Kanocky) have been unable to demonstrate changes, while the degeneration described by others (e. g., Masumoto) is of uncertain significance.

*Peripheral Nerves.*—Meissel treated the skin of white mice, some with 1,000 to 3,000 r generated at 90 kilovolts and others with unfiltered radon of equivalent dosage. As the irradiated skin became hyperplastic, the terminal fibers grew along with it. Nerves involved in radiation necrosis regenerated as though cut.

Doses of 1,000 to 4,000 r have been shown to hinder myelination and finally to destroy completely axons and neurons in the newborn rat (Leboucq).

Exposure of the peripheral nerves of rats to roentgen rays in doses of 4,000, 6,000 and 10,000 r at 200 kilovolts produced no demonstrable histologic or physiologic changes. But complete degeneration of nerves and some evidence of degeneration of the sensory neurons resulted from exposure to 12,000 to 16,000 millicurie hours of gamma radiation (Janzen and Warren).

The excitability of frog nerves has been studied by several workers and has been said by some to be decreased (Audiat and Strohl; Audiat, Auger and Fessard) and by others to be increased (Lazarus-Barlow and Dunbar; Rascanu and others; Redfield and others) after heavy radiation.

*Autonomic Nerves.*—A mild degeneration of neurons of the sympathetic ganglions of the rabbit has been demonstrated following direct exposure to roentgen rays (Suzuki).

The careful studies of Griffith and Pendergrass revealed no evidence that light (165 kilovolts) roentgen radiation permanently or organically alters or destroys the sympathetic ganglions of rats.

Ricker applied 150 mg. of mesothorium to a rabbit's ear for sixty hours and concluded that the hyperemia was the result of the greater sensitivity of the dilator fibers.

Langer observed a certain depressing effect of unfiltered rays in the autonomic nerves "if at the time of radiation the system is overirritated." There was no demonstrable cytologic alteration.

*Summary.*—Peripheral and autonomic nerves are highly resistant to radiation.

#### THE CENTRAL NERVOUS SYSTEM

From the practical angle therapeutic radiation as now generally given has no appreciable effect on nerve tissue. Interruption of the vascular bed rather than direct injury of the nerve elements is probably the cause of the rare nervous symptoms following irradiation of the brain or the cord. A distinction between injury of nerve tissue from radiation and injury from focal ischemia due to damage of blood vessels by radiation cannot always be made. Vascular effects are particularly important in an organ such as the brain, where anastomoses are few.

The pathologic problem is illustrated rather well in 2 patients in whom jacksonian epilepsy developed some time after irradiation of epithelioma of the scalp. One patient, whose case was reported by Fischer and Holfelder, had received 7 erythema doses seven years before epilepsy developed. Biopsy of the brain showed marked changes: loss of cortical structure, loss and alteration of ganglion cells, edema of interstitial tissue and thickening of the intracranial blood vessels, which showed hyaline or amyloid-like change. There was evidence of old hemorrhage and necrosis. The other patient, whose case was reported by Markiewicz, had been given an unknown amount of radiation over a period of five years; nervous symptoms developed one and a half years after the last treatment, and death occurred six months later. In that part of the brain beneath the most severely damaged skin there were multiple necroses and some hemorrhages, most prominent in the white matter. The pial and intracerebral vessels were thickened. Pendergrass, Hodes and Groff reported on 3 patients who died of cerebral infection and thrombosis a few months after receiving heavy doses of radiation.<sup>1</sup> No radiation effect was recognized, but it is possible that minor changes may have been masked by the severe and extensive inflammation.

There are 2 remarkable cases of encephalopathy in childhood in which circumstantial evidence pointed to roentgen rays as the responsible agent. The first case, reported by Lorey and Schaltenbrand, was that of a 5 year old girl whose scalp was irradiated for trichophytosis. Complete epilation occurred within three weeks, and this was permanent on the right side of the scalp. Epileptic attacks and left-sided weakness were noticed a year later. Two years after treatment the child was still suffering from minor seizures, although major ones had become less numerous. Roentgen examination revealed atrophy of the skull and superficial calcification within the cranial cavity. The encephalogram showed the right lateral ventricle wider than the left suggesting atrophy of the right hemisphere. The skull was later trephined, and there was revealed pachymeningitis hemorrhagica (Schaltenbrand). The authors explained the pathologic process as a primary injury to the meninges and vessels which produced a dural hematoma and the train of symptoms—epilepsy, spastic hemiparesis and underdevelopment due to the secondary injury of the cortex.

The second case was that of a 9 year old girl who was treated with radiation for microsporonic infection of the scalp. Pyrexia and bronchial symptoms occurred twice during the interval of treatment. Almost com-

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1. The doses were 21,000 r over a period of six years in case 1, 6,300 r in case 2 and 2,750 milligram hours and 15,200 r at low voltage over three years in case 3.

plete, permanent depilation resulted. The child became emotional and nervous. Six years after treatment, at the age of 15, she was seized with an acute illness characterized by fever, delirium, aphasia, deviation of the head and the eyes to the left, right flaccid hemiparesis and clonic twitchings of the right arm and the right side of the face. Encephalographic examination showed symmetric dilation of the ventricles. Her symptoms gradually improved, and roentgen examination twelve years after treatment showed patchy defects in the cranium and in one place flaky intracranial calcification.

Alpers and Pancoast were unable to find any significant changes in nerve cells in the cerebrum and the cerebellum adjacent to tumors after one or more series of roentgen ray treatments. In 2 cases there was demyelination of the cortex and thinning out of the tangential fibers. In all 5 cases in which cerebellar tumors had been irradiated the Purkinje cells showed mild degenerative changes, such as breaking up of the cell membrane, fatty degeneration of the cytoplasm, loss of Nissl bodies and sometimes loss of nuclei as well. They also appeared less numerous than usual. The authors considered the finding of fat in the Purkinje cells sufficiently uncommon to suggest a possible connection with radiation effect. Fat in mesenchymal and nerve elements was a prominent feature in several cases. One case of some interest showed an abundance of fat in ganglion cells, astrocytes and endothelial cells of blood vessels. In this case there was some thickening of the vessels in the cortex with scattered loss of ganglion cells, often in relation to thickened vessels. The patient was 28 years old, without evidence of systemic disease, and had received only one series of treatments. The only severe reaction was at the site where 25 radon seeds had been implanted after a series of roentgen ray treatments and shortly before death. The changes involved blood vessels, nerve cells and myelin sheaths. There were numerous small hemorrhages, the blood vessels were thickened and the endothelium was swollen. The relatively few remaining nerve cells showed severe injury: vacuolation of cytoplasm, complete loss of Nissl substance and often absence of nuclei. A definite loss of myelin was found in the upper three layers of the cortex, but there was no demyelination in the lower layers. Fat was present in ganglion cells of the cortex, in astrocytes and in endothelial cells, and sometimes its presence was associated with a moderate reaction of granular cells, swelling and chromatolysis of ganglion cells, extensive demyelination in the form of swelling of the sheath, formation of myelin balls and dust-like material, and accumulation of fatty granular cells. A slight progressive change of the Hortega cells throughout the brain and the presence of many ameboid astrocytes in the white substance were also noted.

O'Connell and Brunschwig reported more definite changes in brains which had received roentgen treatment for tumor. The changes were present in parts distant from the tumor and are particularly interesting inasmuch as the vascular changes were said to be too slight to have any influence. The findings in 4 patients are presented in some detail. All the radiation was given at 200 kilovolts and 200 to 300 r was administered at each treatment, through multiple portals. A patient 45 years of age received 12,645 r in six months. Shortly before death two and one-half years later, this patient was given 630 r for gradually developing left hemiparesis. A patient 32 years of age received 15,428 r in approximately equal amounts in two series about nine months apart and over a period of one year and three months. Death occurred one and one-half years after the last treatment. A child 4 years of age received 13,789 r during six months and died ten months later. A child 5½ years of age received 14,229 r in seven months, and death occurred less than a month later. The authors pointed out certain clinical features of interest in connection with the radiation treatment: 1. The usual terminal signs of raised intracranial pressure were absent in the two adults. 2. The patients died in a state of anemia and cachexia. 3. In the 5½ year old child, who was treated for medulloblastoma, death occurred one month after treatment, which is much sooner than usual.

Differential stains brought out in each case abnormalities in nerve cells and glia not always clearly pathologic. Retrogressive changes were present in the nerve cells of the cerebral cortex and to a less extent in those of the cerebellum. Some nerve cells were shrunken and others swollen with the nucleus displaced to the periphery. The great excess of lipochrome pigment observable in nerve cells aggregated into large masses and displaced and apparently sometimes destroyed the neurofibrillae. None of the nerve fibers, medullated or nonmedullated, outside the neighborhood of the tumor were in any way affected. All types of glia cells showed hypertrophy, often in marked degree, but the cells were not uniform in size. Perivascular glial proliferation was conspicuous. The aggregation of lipochrome and clasmatodendrosis were marked in some parts. In addition to hypertrophy of microglia there was some evidence of rod formation and even perhaps of transition to microcytes. The vascular changes were apparently considered of secondary importance, and as they are described it is not at all clear that they showed the usual radiation reaction. In 1 case well marked changes are described as perivascular lymphocytic infiltration, arteriolar hypertrophy, hyalinization of the media and swelling of the intimal coat. Slight medial thickening was mentioned as appearing in other cases.



Perivascular collections of macrophages containing lipochrome were marked in some cases. Another interesting feature was the absence of meningeal reaction except for occasional masses of macrophages.

Scholz and Hsü irradiated the heads of 3 deteriorated schizophrenic patients, 2 of whom, aged 26 and 32, died seventeen and a half and nineteen months after treatment. Six portals were used, with a 180 to 270 per cent erythema dose for each portal, calculated to give an even distribution through the brain of a 400 per cent erythema dose.<sup>2</sup> The administration of each dose was completed in three days. Severe roentgen ray dermatitis followed in each case. The postmortem examination was postponed for days in the first and for months in the second instance. The value of the pathologic observations lies in certain positive findings. Principal among these was the vascular reaction. There were congestion of the meninges and recent as well as old hemorrhage in the meninges, the cortex and the central nuclei. The blood vessels were thickened through hyalinization, swelling and fragmentation of the elastica and, in the regions of necrosis, through infiltration of the walls by inflammatory cells. In one case there was fibrosis of the pia, and in the other, thrombosis of a large pial vein. In the nerve tissue, only acute changes were described with any certainty, such as focal necrosis, occasionally extensive, often associated with hemorrhage.

From these few observations of irradiated human brains it would seem that vascular occlusion may be a serious complication of radiation therapy, whereas primary degeneration of nerve tissue is ordinarily slight. The earliest experimental observations of radiation effect in nerve tissue were in connection with irradiation of the animal as a whole. Mice (Heineke, 1903, 1904; London), guinea pigs (Beaujard and Lhermitte; Heineke, 1903, 1904; Rudis-Jicinsky) and rabbits (Beaujard and Lhermitte; Heineke, 1903; Rudis-Jicinsky) were exposed to roentgen rays (Heineke, 1903, 1904; Rudis-Jicinsky; Seldin) or radium (London), and as a result many died, sometimes with evidence of paralysis. The pathologic change most frequently noticed was intense congestion and hemorrhage. Autolysis (London) and degeneration of nerve cells of the cortex (London) and the spinal cord (Beaujard and Lhermitte; Rudis-Jicinsky) and of the posterior tracts of the cord (Beaujard and Lhermitte, Rudis-Jicinsky) were described, but Heineke (1904) found only chromophilic changes in cerebral ganglion cells in 1 animal. The different manifestations of radiation injury have been described more clearly from later, better controlled experiments. They may be (1) nil, without lesions or symptoms;

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2. The factors were: current, 180 kilovolts and 8 milliamperes; skin-target distance, 50 cm.; dose 28 r per minute (800 r measured in air is taken as a 100 per cent erythema dose); portals, 80 to 150 sq. cm.

(2) symptomatic, either with or without gross and microscopic lesions, or (3) solely cytologic, without symptoms. Some of the negative reports a few days or weeks after moderate use of radiation are not thorough studies and carry little weight (Beier; Krause and Ziegler). Moreover, radiation sufficiently heavy to cause death within a matter of hours or days adds little or nothing to knowledge of histologic effects. Congestion and edema are usually more or less intense. In the parenchyma there is no evidence at all of degeneration (Danysz, 1903b) or there are very slight changes of doubtful significance in ganglion cells of the cortex (Heineke, 1904) and Purkinje cells (Tsuzuki).

The critical dose for the brain of *Macacus rhesus*, when the organ is exposed directly, lies between 3,000 r and 4,000 r at 200 kilovolts (Elsberg and co-workers), but 4,000 r had no effect on the spinal cord.

Not infrequently after radiation treatments there are nervous symptoms, such as sluggishness of reflexes, apathy, tremor, ataxia, paralysis and convulsions. Obersteiner considered that these disorders were circulatory or metabolic in origin. And Balli suggested that the emaciation of some of the paralyzed animals could explain slight changes in many cells. The rather sketchy histologic material at hand more or less supports these views. Certainly it seems exaggerated to lay these symptoms to the minimal degenerative changes in nerve cells or myelin which are usually described. On the other hand, the vascular effects, which are more constant, appear early, and in some instances, are sufficiently severe to cause symptoms.

There is great variability in the degree of vascular change, depending partly on the time the animal is killed. The reaction may be intense without causing any change in the nerve tissue. Scholz (1934) found little gradation of vascular changes in dogs which were given from 4 to 12 erythema doses in a single sitting. Some of the dogs died, and others were killed; the time intervals varied from twenty-four hours to twelve months. Congestion and hemorrhage of the meninges and parenchyma were the chief pathologic findings. A hemorrhagic cyst was found after eleven and one-half months and fresh hemorrhages after six weeks, three months and twelve months. Exposure of the heads of mice to radium in 10 and 15 mg. doses caused, at the end of days or weeks, small hemorrhages in the cerebrum, the cerebellum and the medulla without significant alteration in the nerve cells (Obersteiner). Similar observations were made by Alquier and Faure-Beaulieu after application of radium in therapeutic amounts to the heads and spines of rabbits.

Mogilnitzky and Podljaschuk (1929) described acute vascular changes consisting of congestion, slight swelling of the capillary endothelium and large hemorrhages, without any obvious rupture of vessels, alike in rabbits dying within a few days after 3.5 to 1.5 erythema doses

and in rabbits and dogs killed sixty to eighty-five days after 1 to 3 erythema doses through two or three portals. After the longer intervals of time there were also "thickening" of vessels, appearance of fat in the endothelium, increase in the perivascular glial elements and some thickening of the meninges. An unusual feature was the great variability in the location of the lesions.

Puppies dying in status epilepticus two to seven weeks after administration of 4 Holzknecht units (0.8 erythema dose) in three or four doses showed congestion, edema and sometimes severe focal hemorrhage (Brunner). Congestion of meninges and rupture of capillaries with hemorrhage were the only pathologic effects noted in adult mice that became paralyzed and died ten to forty-four days after exposure to 50 mg. of radium for periods of four hours to twenty days (Danysz, 1903b) or in a dog that became paralyzed five months after 4.5 erythema doses over the lumbar cord (Gabriel).

Evaluating reports of radiation injury to nerve tissue itself is more difficult, partly because controlled observations made by neuropathologists are few. Pertinent factors, such as the conditions of death, the degree of postmortem change and the methods of preparing specimens, are seldom described in sufficient detail. Under these conditions it is almost impossible to evaluate degenerative changes, especially those which are called "slight" and "early."

One of the most authoritative and interesting reports is that of Lyman, Kupalov and Scholz on the brains of dogs studied by Nemenow (1934a) in connection with the effect of radiation on conditioned reflexes. The brains of only 4 of the dogs used by Nemenow (1934b) are described. The roentgen radiation was given through five portals to center in the occipital lobe, so that the posterior part of the head, the cerebellum and the occipital lobe received the heaviest doses. These dogs received 18 to 20 erythema doses, an amount about four times as much as is required to cause some diminution of conditioned reflexes (Nemenow and Kupalov). This dosage was followed by a lowering of the spontaneous activity of the animals for a period of two to five weeks. The reflexes were diffusely inhibited even though the radiation had been aimed at the visual centers of the occipital lobe. Evidence of subcortical reflex change was also presented. "Conditioned reflexes always dropped to a more or less extent and unconditioned secretion might also decrease. The latter suggested suppression of salivary secretion by action of roentgen rays directly on salivary glands." This period of inhibition was followed by normal behavior. One dog (no. 4) that lived longer than the others showed a final stage five to six months after exposure in which signs of cerebral damage were evident, such as ataxia, impairment of vision, circus movements and general deterioration of behavior.

Similar behavior after irradiation of dog's brains has been described by Nemenow and Kupalov and by Warren and Bishop. This dog (no. 4) was the only one which had significant morphologic changes in the brain. Degeneration, although slight, was scattered throughout the cerebral gray and white matter and the optic chiasma. Several large and small fresh foci of necrosis of different sizes were present in the hypothalamus and in the middle section of the corpus callosum. These changes were thought to be largely due to the damage to large and small vessels. The vascular reaction consisted of (a) regressive changes in the capillaries and precapillaries and (b) marked hyaline and sclerotic degeneration of the arterioles in all parts of the brain, especially intensive in the subcortical ganglions. There was also an inflammatory change in the form of slight plasma cell infiltration around blood vessels in the subcortical parts of the brain, chiefly the midbrain. It is interesting with respect to reports of other observers that Lyman, Kupalov and Scholz considered fat in adventitial cells of blood vessels, noted in 1 dog of their series, a commonplace if not an absolutely normal finding in dogs' brains.

Scholz (1934, 1935) described changes in the brains of young and adult dogs varying in severity according to the amount of radiation given and the time of examination afterward. He used roentgen radiation chiefly, but in one experiment radium was used. Acute necrosis, associated with degeneration of vascular endothelium, was found in very young animals three to four weeks after the administration of 1 to 2 erythema doses (human); with 8 to 18 erythema doses focal necrosis and fresh bleeding were found even three months to a year later. In mature dogs 4 to 12 erythema doses apparently did not produce necrosis; a mild inflammatory reaction was described in the white matter, without cell degeneration. More or less intense meningeal hyperemia was present in all dogs. Fresh as well as old parenchymatous hemorrhage was evident a year after 4 erythema doses had been given in one treatment. Vascular fibrosis was noted even in the capillaries; there was also fibrosis of the leptomeninges.

Mogilnitzky and Podljaschuk (1929) found slight degenerative changes in ganglion cells of puppies examined four and a half months after a radiation treatment (1 erythema dose through each of three portals) which in another puppy caused marked congestion, edema and death from convulsions.

The degenerative changes in the brains of dogs that had received 1,500 r or more in one or two doses described by Selle, Westra and Johnson are vague and of doubtful significance. These authors considered the brain more sensitive than the pituitary gland.

Bagg produced severe lesions with filtered and unfiltered radium emanation in small and large amounts. The size of the lesion and the

presence or absence of symptoms seemed to depend on the amount of radium emanation used rather than on the number of millicurie hours. The local reaction consisted of necrosis surrounded by a zone of polymorphonuclear leukocytes plus a zone of hyperemia. Adjacent ganglion cells showed hydropic degeneration and nuclei stained poorly. There were also minute extravasations of blood, as well as perivascular and perinuclear edema. The pia was edematous and contained some round cells. With the larger amount there was, in addition, a more obvious reaction in the vessels, which were necrotic and thrombosed, and the nerve cells in a larger radius showed mild changes. Four thousand or 9,000 millicurie hours heavily filtered was without any effect on the skin or the brain. A monkey whose motor reactions and learning process had been studied and timed before treatment received 2,406 millicuries of filtered radon for five hours (total dose of 12,030 millicurie hours) over the left temporal region and suffered a severe radium burn, but there was no interference with motor reactions. The animal became comatose and finally died on the thirty-fourth day after treatment. No definite gross or microscopic changes were noted in the brain. These experiments demonstrate the caustic local action of beta rays and the resistance of nerve tissue to gamma rays. In later experiments Edwards and Bagg reported lesions in the corpus striatum produced by buried radium emanation.

The degenerative changes observed in monkeys' brains by Horsley and Finzi were apparently dependent on the intense vascular reaction; two platinum tubes each containing 27.7 mg. of pure radium were left for two and one-half to four hours on the precentral and postcentral gyri of 2 monkeys. The examination was made three to four weeks later. Another animal had multiple treatments to different parts of the brain (55 mg. of radium for four hours and 100 mg. for three and one-half and four hours) and was killed on the forty-fifth day after the first exposure, the sixteenth day after the second exposure. The intensity of the reaction was roughly comparable to the dose. The leptomeninges were greatly thickened, with fibrous tissue extending into the sulci and around the vessels; there was marked endothelial proliferation of the blood vessels, in some instances practically occluding the lumens, and the arterioles showed hyaline change. There were minute foci of hemorrhage in the cortex, a few degenerated axons in the pons and, in one animal, small clusters of polymorphonuclears in the outer layers of the cortex.

Focal necrosis with peripheral hemorrhage and hyalinization of vessels but without evidence of repair was seen in the brains of dogs three weeks after 50 mg. of platinum-filtered radium had been placed beneath the dura for four, six, twelve and eighteen hours, respectively (Williamson and co-workers).

Pendergrass, Hayman, Houser and Rambo placed tubes containing radium beneath the dura and inserted radium needles into the parietal lobe in dogs. The doses consisted of 450 to 1,000 milligram hours unfiltered and 1,000 and 2,600 milligram hours of well filtered radium. With a single exception, there were no symptoms when the doses were 1,150 milligram hours or less, and all dogs except 1 receiving 1,400 milligram hours or more died. An increase of 12.6 to 42.8 per cent in the weight of the irradiated hemisphere and an increase of the total nitrogen of the treated side were noted and were explained on the basis of edema. The effect on the animals was so constant that it was possible to predict the time of death within a day or so. The size of the gross lesion depended on the dose and the length of time before death. In one week 500 milligram hours produced no gross lesion. A well defined reddish lesion measuring 2 by 1 by 1 cm. was present eleven days after the administration of 2,300 to 2,600 milligram hours. A scar was found ten months after 360 milligram hours had been given. Softening and cavitation occurred in some instances. The needles produced a more intense reaction, probably owing to the fact that more of the beta rays reached the brain substance.

Judging from experimental data, greater caution would be necessary in irradiating the brains of children than in irradiating those of adults. The reaction of young animals differs in some respects from that of adults; it is more acute, it is caused by smaller doses, and growth is retarded. Danysz (1903a, b) noted that young and old animals given identical treatments with radium had the same symptoms, but that these appeared first in young animals. Dosages of radium or of roentgen rays which had no effect on adult animals caused symptoms in young animals (Danysz, 1903a; Labeau), and radiation just sufficient to produce vascular changes in adult animals may cause degeneration of nerve cells in young animals (Mogilnitzky and Podljaschuk, 1929).

Brunner from his experiments on kittens and puppies with high doses concluded that in the parenchyma of the brain only elements that have a certain lability react, such as the superficial granular cells of the cerebellum, while ganglion cells, myelinated fibers and resting glia are not easily affected. With 2 kittens 11 days old he observed that after one exposure of the head to 4 erythema doses with a 3 mm. aluminum filter the granular layer of the cerebellum at death was thin and in part absent and that there was some increase in glial cells of the cord, as well as focal fibrosis of the meninges.

The inhibitory effect on the growth of the brain has been demonstrated with moderate (3 Sabouraud units, one weekly [Demel]) and heavy doses of radiation (25 erythema doses [Turner and George]). Walter was unable to find cytologic changes in the brains of young animals which were nevertheless dwarfed as a result of radiation injury.

Rachmanow and also Mogilnitzky and Podljaschuk (1930) studied the effect of roentgen rays on the mesenchymal elements of the brain by means of injections of trypan blue in dogs, rabbits and mice. The latter authors reported that trypan blue was present in the vascular endothelium and perivascular glia cells three days after the injections, disappearing in a few days. If a further series of exposures was given, the color reappeared in twenty to thirty days. Rachmanow showed that more endothelial cells and macrocytes vitally stained could be found in the pia of irradiated than in that of nonirradiated mice.

The few studies to demonstrate the sensitivity of the choroid plexus have not been conclusive. Heidrich, Haas and Silberberg found no change in the amount of fluid from three months to a year after exposure of dogs to 9 erythema doses through three portals of entry.

Inaba, Sgalitzer, and Spiegel, also using dogs, found a lowering of the normal curves ten to fourteen days after an 80 per cent erythema dose. Microscopic examination of the choroid plexus revealed pyknotic nuclei and slight changes in the cellular protoplasm. König and Panning found no change in the choroid plexus in rabbits whose heads had been irradiated.

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# Forensic Medicine

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## SEMEN AND SEMINAL STAINS

### A REVIEW OF METHODS USED IN MEDICOLEGAL INVESTIGATIONS

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An investigation of the various gross and microscopic, chemical and immunologic characteristics of semen may be productive of information of both medical and medicolegal importance.<sup>1</sup>

In the realm of law the identification of a given specimen as semen may provide objective evidence of a corroborative character on a charge of rape, criminal or civil, of seduction, of unnatural practices such as sodomy or other perversions, of incest, or of sundry sexual crimes defined by local law and in bastardy proceedings. That such specimens retain their individuality over long periods lengthens the permissible interval between their discovery and use as evidence and their production.

The importance of the detection of semen in cases of alleged sexual offenses has been recognized since antiquity. The first known allusion is found in the Babylonian Talmud. In order to secure a divorce, a man made his wife and a friend drunk, carried the couple to bed and spilled some egg white between them. Then he called witnesses. His accusation was proved to be false by a physician, who identified the true nature of the stain. The script does not say how the stain was proved to be that of albumin. The first scientific paper concerning the medicolegal examination of semen was presented by Orfila, in 1823. Since then this examination has been routinely performed in cases of sexual offense.<sup>2</sup>

## SEMEN

### DEFINITION AND GENERAL CHARACTER

The examination of fluid semen and the examination of dried semen are two distinct procedures which differ in several respects. Semen

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1. The superior figures in the text refer to the footnotes on the individual pages; the numbers listed in the footnotes refer to the references listed in the bibliography. Omitted footnotes correspond to text omitted in the ARCHIVES but given in the author's reprints.

2. 1, 6, 220 a.

produced for examination differs markedly from a specimen accidentally discovered. There are differences also between the ejaculate produced outside the female organs and that recovered from the female genitals.

Male ejaculate is defined as semen regardless of whether all or only part of its constituents are present. Fresh or dried semen may contain no spermatozoa or may be lacking in some of the less characteristic constituents. It contains a mixture of secretions from the testicles, the epididymides, the prostate, the seminal vesicles, Cowper's bulbourethral glands and Littre's urethral glands. It is composed of both fluid and formed elements. The latter are more important than the former for the purposes of identification. Although the cells may be derived from various portions of the urogenital tract, only those from the testicles are characteristic for semen.

#### MACROSCOPIC CHARACTERISTICS

The amount, consistency, color and odor of seminal fluid are neither characteristic nor constant. The quantity of semen is normally from 3 to 5 cc., with an average of 3.3 cc. A smaller amount is often a sign of abnormal composition of the semen; a larger quantity is of little importance. At ejaculation the consistency is gelatinous, but liquefaction occurs within ten to thirty minutes on exposure to air. Semen which is liquid when produced is abnormal; likewise semen which does not liquefy after exposure to air is pathologic. The color is grayish or opaque. The odor may resemble that of chestnut blossoms. Admixture of extraneous protein products, of urine or other material will naturally alter these qualities to some extent. The reaction is alkaline, with a  $p_H$  value of 7.5 to 8.0.

*Normal and Abnormal Variations.*—The macroscopic appearance of fresh semen depends on the age, constitution, health and sexual activity of the male producing it. Generalized diseases, physical or mental, remote localized diseases and diseases of the genitourinary organs will influence the quality of the semen. Such factors as age, frequency of emissions or disease may lower the amount temporarily or permanently below 0.5 cc. and may produce a more liquid consistency. Admixtures of mucus, pus or blood increase the density of semen. Mixed with pus, the specimen may appear yellowish; mixed with blood, red or brown. A greenish color may be caused by contamination of the specimen with *Pseudomonas aeruginosa*.

#### MICROSCOPIC CHARACTERISTICS

The microscopic qualities of seminal fluid undergo various changes. Although not constant, the microscopic aspects are far more characteristic than the macroscopic.

*Number of Spermatozoa.*—The number of spermatozoa in a normal specimen is from 60,000,000 to 120,000,000 in 1 cc. The whole ejaculate should contain at least 300,000,000 spermatozoa. An increase of the number of spermatozoa, called hyperspermia, is not significant, but a reduced number, so-called hypospermia or oligospermia, is regarded as a sign of lowered fertility. The total number of spermatozoa usually decreases with the volume of semen. However, the count may be very low in a voluminous specimen.

*Motility of the Spermatozoa.*—Observations as to the motility of the spermatozoa in a freshly ejaculated specimen provide information of value. The terms "normokinesis," "hyperkinesis" and hypokinesis" refer to the degree of motility. The numbers of normokinetic, hypokinetic and abnormally motile spermatozoa are expressed as percentages of the aggregate. In a normal sample the number of motionless spermatozoa should be less than 15 per cent and that of sluggishly struggling spermatozoa also less than 15 per cent. The causes of immotility are immaturity, old age, malformation, degeneration or disease of the spermatozoa. Under normal conditions 75 to 80 per cent of all spermatozoa should be actively motile. This percentage corresponds to the number of morphologically normal spermatozoa. Some pathologic spermatozoa rotate around an imaginary axis. This may be confirmed by observation of supravitality stained slides.<sup>6</sup>

The motility may be artificially changed in vitro. In samples with subnormal findings the number of actively motile spermatozoa may be increased and the degree of motility of hypokinetic spermatozoa stimulated by the change of the medium. Nonmotile spermatozoa may be induced to show motility if transferred into a suitable medium. The observation of the longevity of spermatozoa in vitro is of little significance in regard to fertility because the findings do not correspond to the behavior of the same spermatozoa in the genitals of a certain female or of women generally. Under appropriate conditions spermatozoa should remain motile for twenty-four hours at room temperature and for twelve hours at body temperature. The motility of spermatozoa aspirated from the female genitals has to be determined in addition to that of spermatozoa in semen produced outside the female organs. The number of the spermatozoa and the degree of their motility do not depend on the technic of ejaculation but may be changed shortly after production by the exposure of the spermatozoa to extraneous influences in vitro as well as in vivo.

*Appearance of the Spermatozoa.*—The cytologic study of semen forms an important part of the evaluation. At least 70 to 80 per cent

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6. 238 b, 239 a.

of all spermatozoa should have a normal appearance. The normal human spermatozoon has an ovoid flattened head, 3 to 6 microns long and 2 to 3 microns wide, with a chromatin-containing nucleus occupying the posterior third of the head. Its cylindric neck shows a centrosome and is linked to the chromatin-containing body with an annular disk. The body ends in a tail which forms the sheath of an axial thread originating from the posterior centrosome.

There is no rule as to the proportions of various atypical spermatozoa, which form up to 25 per cent of the entire number of spermatozoa under normal conditions. Some of the variations are called physiologic ones: 5 to 10 per cent of all spermatozoa may be pear shaped, may have somewhat smaller or larger heads than the majority, or more round or more narrow heads. Another group includes those characterized by a remnant of cytoplasm at the head or neck, appearing as a fine netlike structure and accepting a pale azure color with the Giemsa stain; these spermatozoa represent the immature forms. Normally, 3 to 5 per cent of all the spermatozoa may be immature. The aged forms, showing vacuoles and hyperpigmentation or depigmentation of their heads, represent 2 to 3 per cent of all the spermatozoa of a normal specimen.

Finally, the abnormal spermatozoa may be mentioned. Their number should not exceed 20 per cent of all the cells in a specimen of normal semen. Several hundred types of pathologic spermatozoa have been described. Some of the drawings of various authors resemble artefacts caused by a faulty technic. Some of these types occur rarely, and in practice only about twelve types are important. The abnormalities of the head and neck of the spermatozoa are more common than of those of the body and tail. Some specimens show only a few of these types; in other samples a large variety of atypical forms may be found. A deformed head or neck, a pinhead and a gigantic head are the most common forms. Abnormalities of the acrosome and a thickened head are less common. A double head or tail, a displaced centrosome, an atypically implanted body and finally combined abnormalities are rare. An unusually large head may be seen in the hypermegalospermatozoon, which belongs in the last group. How many of these forms represent malformation, how many degeneration or disease, of the germ cells is not well understood.

*Cells of the Spermatogenesis.*—Normally, only 0.25 to 2 immature cells of the spermatogenesis are present to every 100 ripe spermatozoa. In normal semen only the last stages of evolution preceding the spermatozoal are found. Youthful spermatozoa have a residue of cytoplasm at the neck or head which prevents motility. This residue may be found free among the spermatozoa as a small round pale-colored

body, about 8 to 12 microns in diameter and somewhat granular. Such residual cell forms have been falsely described as spermatids without nuclei. They are the remains of spermatids after liberation of the spermatozoa. They have no importance and normally undergo disintegration.

The metaspermatozoon represents the last stage of evolution preceding the spermatozoal. It is a spermatid with a more or less elongated nucleus measuring about 2 to 3 microns which is about to escape from the plasma and become the nucleus of the future spermatozoon. The nucleus contains much chromatin, the plasma has the same structure as the residual body described in the foregoing paragraph, and the dimensions of the cell are approximately the same as those of a spermatid.

The spermatid is similar; it differs only in that the nucleus is well within the cellular body, being central or somewhat paracentral, more round, and of a diameter of about 2 microns. The plasma is eosinophilic and more uniform. The prespermatid, also called the secondary spermatocyte, is larger, 8 to 16 microns in diameter, and shows three to five or even eight round nuclei, which may be of various sizes or all equally large. Its plasma is lighter and has a netlike structure but no granules. A light retractile zone may be found around the nuclei.

All these cells may be present normally, the more mature ones more often than the less mature ones. Prespermatids are not frequently seen in normal semen. They must not be confused with leukocytes, though they can be differentiated from the latter only by observation of stained smears.

Under abnormal conditions, the total number of evolutionary cells increases, and also more unripe forms appear. There are no limits as to the number of the cellular elements. Under extreme conditions, as in azoospermia, spermatozoa may be absent and only the evolutionary cells may be found.

The spermatocyte, also called the primary spermatocyte, is the largest cell of the spermatogenetic series. It is 12 to 20 microns in diameter; the plasma accepts the azure color of the Giemsa stain; the nucleus is rich in chromatin and stains darkly. All stages of cell division may be encountered. Usually the nucleus fills almost the whole cellular body. Often two solid bean-shaped or round nuclei stay at opposite poles, separated by a central vacuole.

Spermatogonia are rarely seen. The spermatogonium is round, measures 5 to 12 microns in diameter and has a dark eosinophilic plasma and a round or bean-shaped nucleus, occupying about half of the cell or more. Cell division also may be observed.

In addition, all these cells of the spermatogenesis may undergo degeneration; vacuolation, accumulation of the chromatin at the wall of the

nucleus, karyorrhexis, atypical mitosis, amitotic division and finally karyolysis may be observed.

Under pathologic conditions, other elements which have their origin from the sexual glands may be observed. The most characteristic ones are the Sertoli cells. They appear as fusiform cells, 40 microns long and at their widest point 6 to 8 microns wide. In this part the ovoid or kidney-shaped nucleus with a clearly outlined central nucleolus is situated. The nucleus is not solid and accepts a dark violet color; the plasma is finely or sometimes coarsely granulated and stains light azure with Giemsa's stain. Various epithelial cells, which sometimes may merge to form a multinuclear "giant" cell, may have their origin in the various parts of the ejaculatory ducts.

The origin of the macrophages or spermiophages seen is not clear. These cells measure 16 to 20 microns in diameter, are round, have a light eosinophilic plasma with particles of chromatin, and a large semi-lunar eccentric dark nucleus. A vacuole in the indentation of the nucleus characterizes these cells. Usually two to twelve heads of phagocytosed spermatozoa are seen in the cytoplasm. The shape of these heads indicates the time of phagocytosis, the older ones being round, the more recent ones still ovoid. Sometimes a part of the neck of a spermatozoon may be seen just outside the cellular membrane of the macrophage.

*Other Formed Elements.*—Epithelial cells from the urethra characterize present or previous disease. The large polygonal cells, 18 to 24 microns in diameter, of the fossa navicularis urethrae have a light basophilic plasma and a round central nucleus measuring 6 microns. The nucleus contains much chromatin.

Leukocytes are not present under normal conditions. When present, they indicate inflammation of some part of the urogenital organ. Red blood corpuscles are present only under pathologic conditions.

Normally, the fresh ejaculate is free of bacteria. Some organisms may have their origin in the urethra or from the skin. Pathogenic bacteria may originate from any part of the urogenital tract. Protozoa are normally not present. They may be a sign of admixture of infected urine.

Robin's prostatic corpuscles and lecithin corpuscles are described as constituents of normal semen as often as leukocytes are, but are rarely seen as the latter. Just as cells of the spermatogenesis, especially pre-spermatids, are confused with leukocytes, the residual cell forms described in a foregoing paragraph may often be confused with the hyaline prostatic corpuscles. Böttcher's crystals of spermine phosphate appear spontaneously several hours after ejaculation in specimens exposed to air, or more often, only after addition of a 1 per cent watery solution of ammonium phosphate to the semen. They have rhomboid structure

and resemble Charcot-Leyden crystals. Fatty acid crystals and testicular cylinders which resemble small waxy casts are of unknown origin and certainly pathogenic. They are often pictured in drawings but hardly ever seen in practice.

Various elements have been described as constituents of human semen which have their origin from containers. The manner of collection of semen is largely responsible for the microscopic observations apart from the germ cells.

*Azoospermia and Aspermia.*—Azoospermia and aspermia must not be confused. In azoospermia the semen is without spermatozoa but does contain immature cells of the spermatogenesis. Aspermia is a condition of congenital or acquired obstruction of the ducts, and it is characterized by ejaculate without any elements from the sex glands.

Azoospermia may be temporary or permanent. After frequent sexual intercourse or spontaneous emission semen may be free of spermatozoa. Alcoholism, various intoxications or internal diseases may cause temporary or permanent azoospermia. Bilateral cryptorchidism, congenital atrophy or hypoplasia of the testicles, gonorrhea and syphilis, tuberculosis, leprosy and smallpox are the most frequent causes of permanent azoospermia. Many infectious diseases, such as mumps, also may be responsible for this condition. Mental illness is often accompanied by cellular deficiency of the ejaculate.<sup>7</sup>

Aspermia may have similar causes. Bilateral gonorrheal epididymitis, tuberculosis, carcinoma and other pathologic conditions explain acquired aspermia, while embryonal anomalies explain the congenital condition.<sup>8</sup>

The importance of these findings in cases of sterile marriage, questioned paternity and alleged rape was early recognized. Both azoospermia and aspermia are far more frequent than is commonly realized. The latest figures concerning the frequency of semen without spermatozoa among apparently normal men range from 7.2 to 18 per cent. Among 400 men examined for causes of sterile marriage I found 16 per cent to be without spermatozoa. Such high figures represent selected groups of men. For the general population of adult males the lower figures, around 8 per cent, seem more apt to be correct.<sup>9</sup>

Vasectomy does not cause immediate aspermia. For as long as two to three weeks after the operation one may be able to detect active spermatozoa in the semen. The same phenomenon may be noted in the cases of azoospermia and aspermia caused by pathogenic processes. Spermatozoa are stored in the seminal vesicles; hence the gradual disappearance.

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7. 285 a, 288, 331 b.

8. 82, 225 e, 285 a, 291 c.

9. 56, 61, 130, 147, 239 c, 383 c.

*Normal and Abnormal Variations.*—The microscopic composition of semen is even more variable than the macroscopic appearance. Both depend on the same factors: age, frequency of sexual intercourse, relative abundance of prostatic secretion, mental strain, fatigue, activity of the endocrine glands, various debilitating diseases. Race, climate, diet and season do not play any role. They affect potency rather than fertility. Frequent ejaculation causes a decrease of the number of the spermatozoa, affects their motility and changes the spermiogram. Spontaneous emission produces numerous atypical spermatozoa and immature cells. With natural and artificial stimulation of the activity of the testicles, the numbers of youthful spermatozoa and immature cells of the spermatogenesis increase (Pollak and Joël, 1942).

The shift to the left, which corresponds to a similar change in the hemogram, may reach various degrees. The sensitiveness of spermatogenesis to disorders in the organism is high. Under local pathologic conditions the cells of the spermatogenesis may undergo fatty degeneration and vacuolation; in addition, macrophages, leukocytes and degenerated epithelial cells may appear in large numbers. Harmless and pathogenic micro-organisms may be present.

The identification of material as semen may be readily accomplished if spermatozoa are present. Even when spermatozoa are absent, the identification may still be possible by the detection of characteristic immature cells of the spermatogenesis. The absence of spermatozoa may be due to incompleteness of the ejaculation. The ejaculate then consists of only one or some of the constituents of semen. There may be intercourse without emission from the penis. This more commonly occurs in old men and in boys of 13 or 14 years of age. The drop preceding the emission of semen may be free of spermatozoa. Incomplete ejaculation may also develop on a psychic basis.

One should be aware of the fact that sexual crimes are often committed by boys, old men, men suffering from alcoholism and lunatics and that such people frequently have deficient semen. In such cases the number of morphologically abnormal spermatozoa may be unusually high and other changes are more often encountered. In cases in which the material cannot be identified as semen by the morphologic method of demonstrating germ cells, it is necessary to fall back on immunologic methods.

#### TECHNIC OF EXAMINATION OF FRESH FLUID SEMEN

*Requirements Bearing on the Material for Examination.*—Only male ejaculate outside the female genitals can be submitted to a complete laboratory examination. It may be obtained through autoerotic manipulation, through coitus interruptus or, in special cases, on injecting a



convulsive dose of metrazol. The contents of the condom may be unsuitable because of the immobilization of the spermatozoa by chemical and mechanical means, because of changes in the structure effected by lubricants or spermaticidal powders or because of the admixture of extraneous elements. Semen soaked into a cotton plug in the vagina does not furnish reliable results. That obtained in vaginal cups or diaphragms is only slightly better. Material aspirated from the vaginal portions of the genitals at certain periods after cohabitation represents a mixture of semen with the female's secretions. Such material has importance for studies of the motility of the spermatozoa in the female's secretions as compared with their motility in vitro. Samples obtained through rectal massage of the prostate are inadequate. Semen in urine is unsuitable for examination. Fluid obtained by puncture of a testicle renders results different from those obtained with ejaculated material.

The examination should be preceded by a pause in ejaculation of seven days. Only one ejaculation should take place in order to provide material for analysis. The examination should be carried out thirty minutes after ejaculation, but for gross inspection an absolutely fresh sample is requested. Transported material may already have undergone some changes, at least liquefaction. If transported, the specimen should be protected against high temperature. Any temperature higher than the body temperature may harm the spermatozoa. A temperature of 20 C. is the most favorable one, even better than 37 C. A careful family, personal and sexual history of the male and of the female covering as many details as possible and a thorough clinical examination complete the examination of the sperm.

*Macroscopic Examination.*—The gross inspection concerns the volume, consistency, color, odor and reaction of the semen. The ejaculate may be produced into a wide-mouthed graduated cylinder or transferred to a cylinder. An abnormal appearance supports the suspicion of pathologic changes. The total amount should be registered to make it possible to determine the number of spermatozoa in the whole specimen. The reaction may be checked with litmus paper. As no definite conclusions are drawn from the gross examination, the microscopic investigation has to be carried out.

*Sperm Count.*—Various methods are used to determine the number of spermatozoa in the ejaculate.

One cubic centimeter of the liquefied semen may be diluted with 9 cc. of diluting fluid. The simplest diluting fluid is tap water, or physiologic solution of sodium chloride, to 100 cc. of which 0.1 cc. of concentrated carbolfuchsin solution has been added. The diluted semen is drawn to the 0.5 mark of a pipet for erythrocytes and the diluting fluid to the 101 mark. Thus the material is diluted 1:2,000. The count is carried out in the inner four hundred small (twenty-five large) squares of a Neubauer counting chamber. The sum of spermatozoa counted

in these four hundred small squares divided by 2 expresses the number of spermatozoa in millions in 1 cc. of semen.

*Motility Test.*—After complete liquefaction of the semen, a drop is placed between a slide and a cover glass, and 100 spermatozoa are observed for motility. The number of normokinetic, of hypokinetic, of abnormally moving and of nonmotile spermatozoa is registered and expressed in percentage. The observation is carried out under the high dry power lens. Ehrlich's ocular screen, which divides the field of vision into four quadrants, may be used to make the studies easier.

Among the various solutions used to stimulate spermatozoa to maximum motility and to revive nonmotile ones, a mixture of 8 parts of a 5.43 per cent dextrose solution with 2 parts of an eighth-normal solution of magnesium hydroxide is the best.

The longevity in vitro may be observed in hanging drops under a special cover glass, perforated to permit the access of oxygen, or in open small test tubes. Specimens may be kept at room (20 C.), scrotal (35 C.) and body (37 C.) temperature. Various investigators perform special tests, exposing the spermatozoa to temperatures of from 41 to 55 C., to hypertonic and hypotonic, acid and alkaline solutions, and to lactic acid, changing the  $p_H$ . The value of these tests is much discussed, and no definite conclusions can be drawn as yet. In view of the differences between the observations made in vitro and those made in vivo, their value seems rather limited.

*Methods of Morphologic Studies.*—The structure of spermatozoa may be checked by observation of the organisms in hanging drops with addition of a drop of a 1 per cent watery solution of brilliant cresyl blue. A second method involves the preparation of thin films of liquefied semen. These are prepared in the same way as blood films, speedily dried by fanning in the air, and fixed for three minutes with absolute methyl alcohol. Finally, the sample may be centrifuged, hardened in solution of formaldehyde U. S. P. and in a sequence of alcohol, and prepared for embedding in paraffin by passing the material through a sequence of alcohol with xylene; the sections may be stained with iron-hematoxylin and eosin. Various methods of coloring are recommended for the fixed smears. One slide may be stained for twenty minutes with freshly diluted Giemsa-Romanovski azure-eosin solution (to 5 cc. of distilled water 10 drops of the dye are added) and the smear washed then with distilled water. Another slide may be stained with hemalum (5 cc. of a 2 per cent solution of crystalline hematin in 95 per cent alcohol and 100 cc. of a 5 per cent watery solution of potassium alum) for twenty minutes, steeped in running water for thirty minutes, colored again with hemalum for five minutes, rinsed in water for ten minutes, colored for contrast with 3 per cent alcoholic solution of eosin for three minutes,

differentiated in 70, 90 and finally 100 per cent ethyl alcohol, cleared in carbolxylene and embedded in balsam under a cover glass.

*Biometrics and the Spermiogram.*—By projecting spermatozoal heads on a screen in a darkened room their size may be magnified three thousand times and can be measured. A biometric curve may be drawn. This curve is more or less characteristic for each individual. The number of spermatozoa measured must exceed 300. In the evaluation of the curve all conditions changing the structure of the germ cells must be

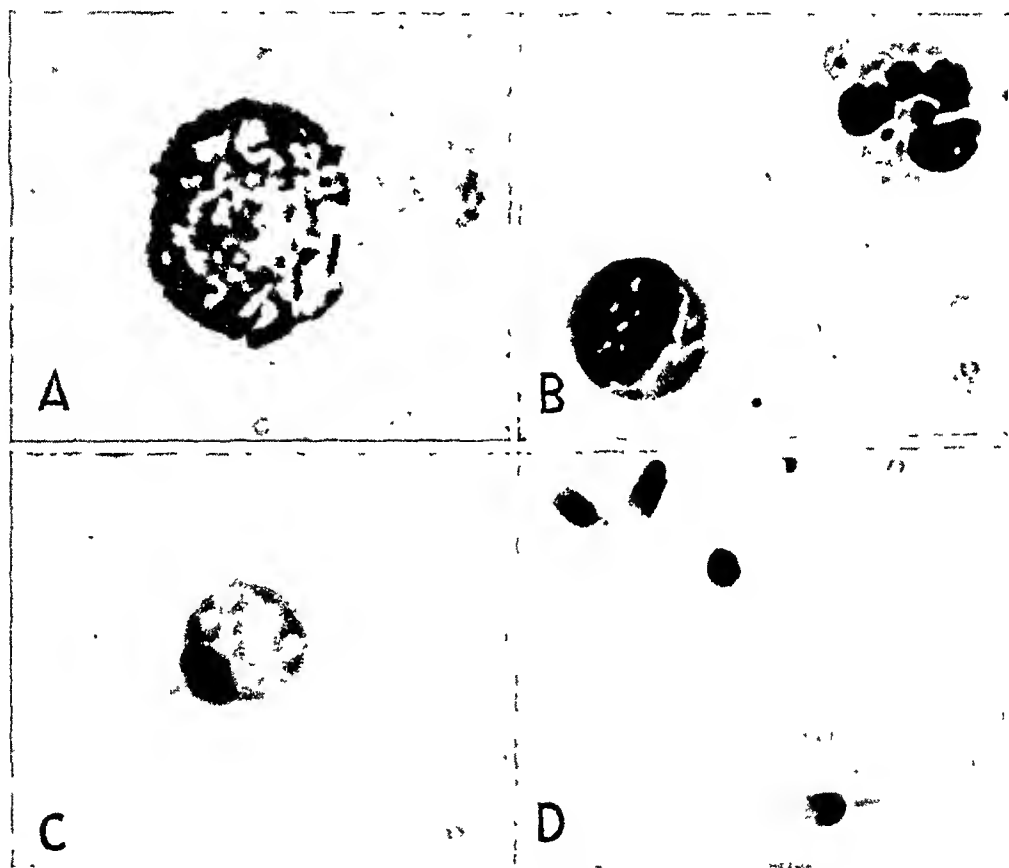


Fig. 1.—Some of the cells seen in seminal smears: *A*, giant cell; *B*, spermatoocyte and multinuclear spermatid; *C*, escape of the head (metaspermatozoon); *D*, spermatozoa. Giemsa's stain;  $\times 510$ .

taken into consideration. In spite of these limitations, studies of biometric curves of fresh semen samples may provide useful information in establishing individual identity (Mönch, 1934 *a* and *c*).

The spermiogram completes these studies. It is of special value in cases of disputed paternity, questioned fertility and divorce. It furnishes information about the sexual life and about the function and state of the sex glands, and also informs about the condition of the whole body. Various spermiograms have been recommended. Some investigators register only the various types of spermatozoa; others tabulate

all microscopic elements. Again at least 300 cells are studied in stained films. The cells detected are recorded on a chart. I present here my own form of spermiogram.

### SPERMIOGRAM

#### A. Normal Cells of the Spermatogenesis

1. Spermatogonia
2. Spermatocytes (primary)
3. Prespermatids (secondary spermatocytes)
4. Spermatids
5. Metaspermatozoa
6. Youthful, immature, unripe, nonmotile spermatozoa
  - (a) Cytoplasm rest at the head
  - (b) Cytoplasm rest at the neck
7. Mature, ripe, motile spermatozoa
  - (a) Normal spermatozoa
  - (b) Physiologic variations
    1. Round head
    2. Narrow head
    3. Tapering, piriform, rejuvenated head
    4. Small head
    5. Large head
8. Aged, hypermature, overripe, nonmotile spermatozoa
  - (a) Vacuoles in head
  - (b) Hyperpigmentation or depigmentation of head

#### B. Abnormal Cells of the Spermatogenesis

1. Degenerative immature cells
  - (a) Vacuoles (vacuolation)
  - (b) Fatty degeneration
  - (c) Hyaline degeneration
2. Degenerative? teratoid? diseased? abnormal spermatozoa
  - (a) Abnormal head
    1. Asthenic head, pinhead, microspermatozoon
    2. Gigantic head, megalospermatozoon, macrospermatozoon
    3. Deformed head
    4. Abnormities of acrosome, atypical colored head
    5. Double head
  - (b) Abnormal neck
    1. Thickened center
    2. Deformed center
    3. Abaxial implantation of the center
  - (c) Abnormal body and tail
    1. Displaced centrosome
    2. Naked, sheathless center thread
    3. Double tail
  - (d) Combined forms and rare forms

## C. Other Cellular Elements from the Sex Glands

1. Sertoli cells
2. Epithelial cells from ejaculatory ducts
3. Residual cell forms

## D. Other Cells

1. Leukocytes
2. Erythrocytes
3. Epithelial cells from urethra
4. Spermiophages
5. Protozoa
6. Bacteria
7. Molds

## E. Other Formed Elements

1. Prostatic corpuscles, corpora amylacea
2. Lecithin corpuscles
3. Fat crystals, acids
4. Böttcher's crystals
5. Testicle cylinders

## F. Extraneous Elements

EXAMINATION OF SEMEN RECOVERED FROM THE  
FEMALE GENITALS

## FATE OF SPERMATOOA IN THE FEMALE GENITAL TRACT

The examination of material recovered from the female genitals does not permit adequate judgment as to the macroscopic characteristics of the semen. Some of the semen is lost, and mixture of the remainder with female secretions results in an alteration of the character of the recovered material. Furthermore, the microscopic picture of this material differs from that of semen produced outside the female genitals. Vulvar, vaginal, cervical and uterine cells, bacteria, mucus and even foreign substances are likely to change the morphologic characteristics. Agglutination and phagocytosis of spermatozoa may take place. The chemical environments provided by the various portions of the female genitals alter the motility and the structure of the spermatozoa.

The fate of spermatozoa in the female genitals is incompletely known. Spermatozoa move or are moved out of the vagina, through the uterus and into the fallopian tubes. In addition, lysis, resorption, phagocytosis or active penetration of the spermatozoa into the epithelium may occur. It is not known in which parts such processes occur, and it is not known how many spermatozoa are removed by one or the other of these several processes.

The  $p_H$  of the medium does not affect spermatozoa as much as is commonly thought. At least, *in vitro* human spermatozoa are highly resistant to organic and inorganic acids. Normal semen contains both acid-resistant and alkali-resistant spermatozoa. Antibodies and specific spermatotoxins and lysins, though often discussed, do not seem to be important. The discussion is not closed (Jöel and Pollák).

Material may be secured from the vagina by a platinum loop, a pipet or a vaginal flushing apparatus and from the cervix by a loop, pipet, a glass syringe or a special suction cup. Besides the disadvantages mentioned, the material taken from the female genitals cannot be used for biometric and other studies which would lead toward the identity of a certain person. Two or more specimens of semen from one man or from different men may be mixed together. I had the opportunity to examine an 11 year old girl who admitted that during an afternoon she had indulged in sexual intercourse with 4 men. Her 9 year old friend had intercourse with 5 men within the same length of time.<sup>10</sup>

The motility of the spermatozoa may persist longer *in vitro* than *in vivo* in some cases, while in other cases it may last longer in the female genitals than on the slide. The motility in the vagina and in the cervix is not the same. An examination of spermatozoa from the vagina yields no practical information. Material from the cervix is investigated in cases of questioned fertility (Weisman, 1941).

Spermatozoa which are motile in semen at ejaculation may be immobilized by the cervical secretion, or their motility may be increased. The motility may last six times longer in the cervix than on the slide. Semen of one and the same man used for artificial insemination showed different results in 5 women: In 1 case immobilization of the spermatozoa took place after one hundred and ten hours, in 2 cases after forty-five hours, in one case after three hours and in 1 after three minutes. The motility of spermatozoa in the cervix lasts longest between the fourteenth and the eighteenth or nineteenth day after menstruation. The character of the secretions and of the cells of the cervix changes with the sex cycle, and the motility of the spermatozoa may vary accordingly.<sup>11</sup>

#### PRESENCE AND VITALITY OF SPERMATOZOA IN THE VAGINA

When spermatozoa are found in the female organs, the question is often presented as to how much time has elapsed since the sexual intercourse which produced them. The various sectors of the female genitals are studied separately.

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10. 131 *d*, 194, 331 *b*.

11. 40, 44 *a* and *b*, 45, 275, 281 *a* and *b*.

Spermatozoa have been detected in the vagina at varying periods following intercourse. The findings of various investigators vary from forty-five minutes to seventeen days. Earlier studies give longer periods than the more recent ones. The smaller figures are based on more elaborate observations.<sup>12</sup>

In determining how long before examination the last intercourse occurred, the survival time of spermatozoa in the vagina is of controlling significance. Spermatozoa discovered in other parts of the genitals may be vestiges of still earlier sexual activity. After intercourse, some of the spermatozoa reach the upper parts within a short time, while others remain in the vagina. According to various observers, spermatozoa in the vagina retain their motility for from thirty minutes to twenty-eight hours. The number of motile spermatozoa discoverable in the vagina may be normal after one hour and markedly decreased after two hours; after three hours usually no motile spermatozoa are present.<sup>13</sup>

Menstruation often prolongs motility in the vagina to as long as four hours, compared with the normal period of thirty to forty-five minutes. A change in the reaction of the vaginal secretion after cohabitation could not be detected by one but is reported by others. The  $p_H$  of the vagina one hour after coitus is the same as before, i. e., 8 to 9, but two hours after coitus it is 7 to 8 and three hours after coitus 5.5 to 7.5. These changes may be chiefly responsible for the immobilization of the spermatozoa, whereas the temperature in the vagina and the consumption of dextrose play a less important role.<sup>14</sup>

#### RATE AND CAUSE OF THE MIGRATION OF SPERMATOZOA

Spermatozoa first appear in the upper part of the cervix and in the uterus within three to four minutes after coitus. Belonoschkin explained this phenomenon: It takes place in the case of orgasm only and is caused by the aspiration of semen into the upper part of the genital tract. In the case of active migration of spermatozoa unaided by orgasm, the time required to reach the same sites varies from forty minutes to two hours (average, one hour). This datum on the migration of spermatozoa represents only one of the numerous theories which are based on experiments with animals performed by various investigators. Active chemotactic penetration of the spermatozoa into the cervix seems less probable than their direct ejaculation into the cervical os or the formation of a seminal pool in the posterior vaginal cul-de-sac. Various theories try to explain the mechanism of aspiration of semen into the uterus. The

12. 13 *b*, *c* and *d*, 16 *a* and *b*, 30, 75, 114 *b*, 122 *a*, 127, 131 *b*, 191 *b*, 203 *b*, 264, 294, 309, 323.

13. 127, 131 *a*, 155 *a* and *b*, 225 *a* and *b*, 230, 278, 331 *a* and *b*.

14. 121, 127, 331 *b*.

lack of orgasm may explain the fact that in cases of artificial insemination spermatozoa are found in the uterus twenty-five or thirty minutes after injection instead of within five minutes as under natural conditions. One has to keep in mind that in cases of rape orgasm hardly ever occurs.<sup>15</sup>

The period during which spermatozoa may be found in the cervix after intercourse is variously stated by the investigators as from six hours to seven days.<sup>16</sup>

Again, the motility of spermatozoa in the cervix is of separate interest. The period during which the spermatozoa may be found motile in the cervix after intercourse is given as from forty-eight hours to seven and a half days. Mönch explained the detection of motile spermatozoa after several days: These spermatozoa are not motile in the genitals but regain their motility on exposure to oxygen in vitro. One must not confuse death and temporary nonmotility of spermatozoa; one must also differentiate between motility and fertilizing ability.<sup>17</sup>

The period of motility of spermatozoa is shorter in the uterus than in the cervix—twenty-four to forty-eight hours compared with forty-eight to one hundred and ten hours.

#### DETECTION OF SPERMATOZOA IN THE FALLOPIAN TUBES

The arrival of the spermatozoa at the uterotubal ostiums probably takes place within twenty to twenty-five seconds after cohabitation at the earliest and usually within two to ten minutes. The spermatozoa reach the distal end of the tube within twenty to thirty minutes at the least or within four to five or six hours at the most (Weisman, 1941).

In 1 case motile spermatozoa were found in a fallopian tube nine days after hospitalization of the patient and possibly two and a half weeks after the last intercourse. In 2 other cases spermatozoa were demonstrable in the tubes two weeks after sexual intercourse. Contrary to such older reports, in the tubes of 5 women examined two and a half days to five days after copulation no spermatozoa were found. It is believed that as a rule they may be detected there only up to forty-eight hours.<sup>18</sup>

The many interesting experiments carried out on animals of various species cannot be used as a basis for conclusions regarding human beings. There is hardly another field of physiology in which the differences between the various species are as impressive as in the sexual field, including the behavior of spermatozoa. This is the reason why only

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15. 16 *a* and *b*, 274, 278.

16. 16 *a* and *b*, 44 *b*, 127, 131 *b*, 264, 276.

17. 30, 44 *a*, 131 *b* and *c*, 136, 204 *d*, 331 *b*.

18. 16 *a* and *b*, 76, 215, 217, 331 *b*.



findings concerning human spermatozoa are mentioned in this paper. Concerning human spermatozoa, Weisman in his book summarizes the periods of motility as follows:

Vagina .....	2 to 3 hours
Cervix .....	48 to 110 hours
Uterus .....	24 to 48 hours
Fallopian tubes .....	24 to 48 hours
In vitro at:	
50 C. ....	1 hour
37 C. (body temperature).....	10 to 12 hours
20 C. (room temperature).....	30 to 48 hours

PERIOD DURING WHICH SPERMATOZOA ARE RECOGNIZABLE  
AFTER THE FEMALE'S DEATH

The presence and survival of spermatozoa depend on local and general factors. Physiologic and pathologic conditions, the general health of both the male and the female, the quantity and the quality of the spermatozoa and the chemism of the vagina and the cervix all have an influence on the motility of the spermatozoa and on their preservation after loss of motility in the female genitals.

Only a few reports concerning the detection of spermatozoa in the dead female's genitals are available, and they are inaccurate. Spermatozoa were found in the vaginas of 2 of 3 prostitutes, all of whom had engaged in sexual intercourse shortly before violent deaths. In 1 instance motile spermatozoa were detected in the fallopian tubes fifteen hours after sexual intercourse; the case reported was that of a prostitute who died with her partner during the act of copulation as a result of poisoning with carbon monoxide.<sup>19</sup>

The assailant often undertakes active or passive cleansing of the victim. The surviving female may cleanse the parts even more thoroughly than if the man tried to remove the ejaculate. In a certain case a man stuffed the vagina of his victim with moss in an effort to disturb the detection of semen. In another case lavage with Cologne water made it impossible to detect spermatozoa in the vagina. Washing with water and soap may prevent the examiner's obtaining positive results even though the vagina is searched promptly after such cleansing.<sup>20</sup>

Lapse of time prior to the discovery of a dead victim and artificial removal of the material are two main factors rendering the results of examination less favorable than during life. Except for these factors, the findings may be the same. The conditions affecting the survival

19. 128, 344.

20. 13 b, 207.

and presence of spermatozoa at the moment of death may be decisive for the results of the analysis.

As stated before, only the findings in the vagina may be used for the reconstruction of the time elapsed after the last copulation. Although the various authors give the time of survival of spermatozoa in the vagina as from thirty minutes to twenty-eight hours and the period of their presence in the vagina as from thirty minutes to seventeen days, one may safely consider the periods to be, for motile spermatozoa in the vagina after coitus, thirty minutes to two or three hours, and for non-motile spermatozoa in the vagina after coitus thirty minutes to twenty-four hours.

### SEMINAL STAINS

#### MACROSCOPIC CHARACTERISTICS OF DRIED SEMINAL STAINS

*Material for Examination.*—In practice the identification of dried stains as of seminal origin may be rendered difficult by the nature of the material on which the stain is formed. Laboratory experiments with artificial stains cannot be compared with the analysis of material as obtained in office practice. Urine or feces, urethral, vaginal or cervical discharges, pus from the genitals or from furuncles, blood from insects, menstrual blood, other blood, sweat, tears, nasal mucus, saliva, sputum, gastric contents, stains from fruits or vegetables, from sauce, coffee, tea, beer or wine, from drugs, such as silver nitrate and lead acetate—any of these may be combined or mixed with semen stains. The picture of what one may discover is therefore infinitely variable. In 1 case rape occurred in a barbershop: three types of stains contained various kinds of hair, semen mixed with blood, and vaginal cells. In another case of rape a shirt showed fourteen various stains, six of which were identified as semen mixed with blood. The aspect of a stained cloth buried in a garden cannot be imitated in the laboratory. The gross appearance of semen stains depends on the material stained, on climatic factors, such as temperature and humidity, and even on the presence of micro-organisms and insects. Little is known about the digestion of spermatozoa by maggots. In connection with this question findings concerning the action of artificial gastric juice on spermatozoa are of interest: Spermatozoa are immobilized within five to ten hours; their tails are digested within two days and their heads within four days. In order to prevent further damage of the material beyond that present when the stain is discovered, persons handling the material are advised to protect it against moisture, infection and friction.<sup>21</sup>

*Number, Size, Shape, Color, Consistency and Odor of Stains.*—The number and the size of seminal stains depend on their origin. In the case

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21. 13 a, 49, 101, 212 d and f, 225 d, 259 a.

of spontaneous emission there is usually only a single stain some 7 cm. in diameter. Material ejaculated outside the female genitals at an interrupted coitus usually forms several smaller irregular spots, roughly from 0.5 to 3 cm. in diameter. A distribution of the stains in ring form suggests that the cloth was used for cleansing of the penis. An arrangement of the stains in stripes is more likely due to cleansing of the female genitals or other objects.

The outline of seminal stains is maplike, the border sinuous and deeper stained because of the concentration of chemical constituents.

The color is gray, sometimes yellowish gray or yellow, or occasionally reddish owing to admixture of pus or blood.

The consistency depends on the material on which the stain occurs. On nonabsorbent material, such as stone, marble, metals, glass, or on skin, leather, rubber, fur, velvet, heavy woolen cloth, starched linen, rayon or cotton, the stain has a grayish scaly, starched, gummed or collodium-like appearance. On absorbent material, such as some textiles (cotton, silk, linen) or sponges, the stain is colorless, hardly visible. Testing of the consistency by touch is an important step in the examination. Such factors as the color of the stained material, its structure or its admixture with other substances may change the appearance of the stain.<sup>22</sup>

Moist seminal stains have an odor similar to that of chestnut blossoms, but this odor seldom develops in dried stains after application of moist heat. Some believe that the odor described is characteristic or typical for semen furnished at intercourse, but pus, nasal mucus and other dried or heated organic material may have a similar odor. Odor is not important for the identification of stains.<sup>23</sup>

The macroscopic examination of seminal stains does not seem reliable. None of the characteristics is typical solely of semen. Indeed, almost all human secretions have certain attributes in common. Not only do different samples of semen produce somewhat different stains, but the same ejaculate may appear different on various backgrounds. One and the same person may furnish variable semen. The unreliability of the gross examination of seminal stains is obvious, but still the testimony of laymen based on macroscopic inspection of stains is being held competent by some courts.<sup>24</sup>

*Detection of Stains by Special Illumination.*—Various methods have been proposed for the detection of stains. The stained portions of a cloth may be somewhat translucent, its meshes being more or less filled with material. In such a case viewing the cloth by transmitted

22. 87 *a* and *d*, 195, 196, 266, 268, 341.

23. 13 *c* and *d*, 87 *a* and *b*, 191 *b*, 212 *e*, 254, 269, 294, 313, 341.

24. 46 *a*, 71 *a*, 139, 263 *a* and *b*.

light may be helpful. Carbon light, in which albumin appears deep yellow, semen and other substances pale yellow, and Auer's light have been applied. In filtered Wood's light seminal stains and urine give a white-bluish fluorescence. Ultraviolet rays may be filtered through cobalt or nickel oxide. The fluorescence changes with the type of material stained and is not specific for semen. It is due to the choline content of semen and disappears if the stain is soaked in water. In spite of these limitations, the method is often recommended. It was improved by measuring the wavelength and intensity of the fluorescence. Fluorescent light between 4,000 and 4,900 angstroms, with a maximum around 4,200 millimicrons, is supposedly characteristic for semen, regardless of the age of the stain and independent of the spermatozoa. The contrast on spectrographic photographs may be increased with a 10 per cent solution of potassium bromide. The statement that this method is indispensable for the identification of stains as those of semen does not seem justified. The method is, however, of high value for the localization of stains.<sup>25</sup>

*Differentiation of Stains by Special Chemicals and Dyes.*—Various reagents have been used for the differentiation of stains. The results depend on the presence or absence of albumin. Albumin, which contains sulfur, can be proved present or absent by application of lead compounds. Ammonium molybdate dissolved in nitric acid stains seminal spots deep yellow owing to the presence of phosphorus. Heat, and also vapors of tincture of iodine, color seminal stains yellow or brown.<sup>26</sup>

The first dyes used for the discovery and identification of seminal stains were carmine in ammonia, or a mixture of ammoniacal carmine with trinitrophenol, a solution of erythrosin, potassium bichromate and sodium sulfate in ammonia. The material was first colored and then decolorized with a solution of sodium carbonate. Seminal stains should resist decolorizing much longer than other biologic matter. Claims of specificity of the results for seminal stains do not withstand criticism.<sup>27</sup>

*Identification of Stains by Chemical Reactions.*—Seminal stains have been stated to be characterized by the following attributes: (1) their yellow color which disappears under application of distilled water, (2) their odor, (3) the gelatinous flocculation of the filtered extract and (4) their chemical reactions. Alcohol, lead acetate, lead subacetate and mercury bichloride when added to the extract of a seminal stain furnish a white precipitate, while nitric acid causes the extract to turn yellow. These criteria have been widely accepted by elder investigators. Tests for albumin with nitric acid, with glacial acetic

25. 5, 57, 87 a, b and d, 126, 132, 135, 159, 186 a, 213 g, 240, 277, 281 b, 282.

26. 87 a and d, 129, 159, 254.

27. 31 a, b and c, 34, 59 a and b, 115, 229, 326 a and b.

acid and with potassium ferricyanide, as well as the xanthoproteic reaction, are negative with extracts of seminal stains. The biuret test is positive; Millon's test may be positive or negative. None of these tests gives a positive result characteristic for semen or suffices to exclude semen if negative.<sup>28</sup>

*Comments on the Macroscopic Examination of Stains.*—The most important step in the gross examination is the detection of the stains and the coordination of their distribution with the circumstances noted at the scene of the crime. The detection of the stains may be facilitated in two ways: 1. Various illuminations may be used, among which that from ultraviolet rays has the most advantage. 2. Chemicals or dyes may be used, but among the recommended ones only ammonium molybdate in nitric acid achieves good results. The acid interferes with further examination of the stain. Therefore this method cannot be recommended. A 0.01 per cent solution of sodium sulfate of alizarin in distilled water turns violet any stains caused by an alkaline substrate. Other indicators may be used as well. Seminal stains always give the reaction, as semen is alkaline. Watery extracts of seminal stains may be tested with nitrazine paper and compared with a chart.

Neither ultraviolet rays nor alizarin helps to differentiate between semen and other material. They merely facilitate the detection of stains. They do not interfere with the further analysis of the material. Other chemicals and dyes recommended for use in the identification of a stain as semen do not yield satisfactory results, nor do the macrochemical tests employing extracts of stains. The value of the spectrographic examination may be lower in practice than in theory because the material available for examination is often unclean. A reliable method for the macroscopic identification of seminal stains is not yet known.

#### MICROSCOPIC CHARACTERISTICS OF SEMINAL STAINS

*Cells and Fluid; History of Finding of Cells.*—Semen consists of two parts which can be separated by centrifugation: fluid and cells. Seminal fluid contains 80 to 90 per cent water, 1 to 2 per cent salts, 8 to 10 per cent organic matter, 2 to 6 per cent proteins and 0.21 per cent lipoids. Seminal plasma contains 150 to 175 mg. of phosphorus, 24 to 25 mg. of calcium, 200 mg. of sodium chloride, 200 to 300 mg. of dextrose, 72 mg. of urea, 90 to 100 mg. of lactic acid and 80 mg. of cholesterol per hundred cubic centimeters. None of these chemical constituents is found solely in semen. Compared with blood plasma, the plasma of semen contains one or two hundred times more phosphorus, two and a half times more calcium, twice as much sugar,

28. 4, 13 c and d, 33, 54 a and b, 87 a, b and d, 129, 152 a and b, 171, 195, 220 b, 221 a, 242 a, 245, 254, 269.

twice as much urea, four to five times as much lactic acid, and half as much cholesterol. The identification of a liquid sample as semen by a quantitative chemical analysis may be possible. Dried seminal stains, however, cannot be identified in this way. In addition, one has to differentiate semen not only from blood but also from other biologic material of a more similar chemical composition.

The cellular elements may be divided into two groups. One group is formed by germ cells; the other, by cellular elements of various origin. The presence of germ cells is typical for semen, and this fact permits identification of both fresh and dried samples by morphologic means. The presence of spermatozoa in a spermatocele or in fluid obtained from the testicles, the epididymus or the seminal vesicles by puncture has only theoretic importance. Commonly, the detection of spermatozoa is tantamount to identifying the specimen as semen.

Orfila, in 1827, was the first one to use the microscope for medico-legal purposes, though this distinction has been claimed by others. He employed the microscope to search for spermatozoa in stains. His first attempt did not meet with success, but later in a discussion, Orfila claimed priority in the detection of spermatozoa in stains. The misunderstanding arose owing to the change of the text in successive editions of textbooks. The truth is that the first successful recovery of spermatozoa from stains was made by Lebailiff.<sup>29</sup>

Many investigators have concurred in holding that the detection of spermatozoa is the only proof that a stain is that of semen. Their contention is generally accepted. One must be conscious of the fact that semen may be without spermatozoa. Further difficulties may be introduced by external factors, especially by partial removal of the stain.<sup>30</sup>

*Cellular Artefacts in Stains.*—The fundamental desideratum is the detection of at least one unharmed spermatozoon with a normally outlined head, neck, body and tail. Spermatozoa may be damaged or broken and their parts separated. This may be due to external factors or to handling during the laboratory investigation. The detection of isolated parts of spermatozoa is not significant for diagnosis. Fibers of the cloth, bacteria, trichobacteria and molds may suggest tails of spermatozoa. Yeast, yeastlike fungi, certain varieties of *Monilia*, cells from the urinary tract or their nuclei, blood cells and spores of bacilli may suggest heads of spermatozoa. Some infusoria, cercarias, *Trichomonas vaginalis* or *intestinalis* and certain bacilli (*Trommelschlägelbakterien*) have to be differentiated from spermatozoa.<sup>31</sup>

29. 46 a, 54 b, 71 a and b, 72, 163, 220 b, c and d, 327.

30. 74 a, 82, 106, 144, 303.

31. 87 a and d, 124, 282.

*Trichomonas Vaginalis, Monilia, Bacteria, Pus and Vaginal Cells in Stains.*—Confusion of *Trichomonas vaginalis* with spermatozoa is hardly possible. Its longitudinal diameter is twice or three times as long as the length of the head of a spermatozoon; it may be as wide or twice or four times as wide as the head of the spermatozoon. The terminal flagellum is short, and no center link is seen between the body and this flagellum. Often the four short flagella at the front are also seen. The nucleus is commonly visible, especially when stained. The slower movement and the rotation also distinguish the protozoon from the spermatozoon. Only exceptionally a giant spermatozoon may show the dimensions of a trichomonad. The detection of *T. vaginalis* in a stain on a man's clothing is erroneously considered to establish a significant female origin of the stain. In fact, however, this organism may be the cause of cystitis in the male, and may be present in male urine and stains. The same is true of intestinal flagellates. The detection of *T. vaginalis* in a stain acquires great significance only when the organism has been detected in the complaining female and has been found simultaneously absent from the suspected assailant. Motile trichomonads were found under the foreskin of an assailant six hours after the crime. This contributed to the conviction of the accused man.<sup>32</sup>

The confusion of *Monilia albicans* and other types of *Monilia* with the heads of spermatozoa hardly ever occurs. The head of the spermatozoon is four times larger. Spermatozoa only seldom, as in the case of hyperpigmentation or abnormality of the acrosome, show a uniform stain, whereas the conidium of *Monilia* accepts only one color. The more characteristic mycelium is usually seen beside the conidium.

Rules similar to those for the evaluation of protozoa and molds are valid for the discovery of bacteria in stains. In a case of rape of a 7 year old girl, stains found on the clothing of the girl and of her suspected assailant could not be identified as those of semen. As the girl was infected with gonorrhea by the assailant, a negative result from bacteriologic examination of the suspected man was strong proof of his innocence. I experienced a contrary case in which the evidence could be based on a strong positive complement fixation test for gonorrhea in the case of the assailant.

Pus may be of genital or extragenital origin and from the male or from the female. A pus stain on a man's shirt may have some importance as evidence if the assailed female has a purulent discharge while none is found in the suspected male.

A study of smears may lead to important conclusions. The proportion of extracellular and intracellular gonococci and of pus, vaginal cells and mucus may be used for the estimation of the interval of time

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32. 12, 53, 128, 184, 254, 258, 340.

between infection and examination. In a case of recent gonorrheal infection of an 11 year old girl, I was able through this method to aid in the reconstruction of the assault and to procure conviction of her assailant.

The female origin of a stain can be proved by the detection of vaginal cells. The differentiation of vaginal cells from other cellular elements may be achieved by extraction of the discovered stain in absolute alcohol and staining of slides with Best's carmine for glycogen. In cases in which a cloth has been used for wiping out the vagina, these cells are always present. The appearance of vaginal cells changes with the cycle, and the findings may render some additional information. The detection of vaginal cells together with spermatozoa excludes the possibility of spontaneous emission of the semen which caused the stain. Pus cells containing glycogen hardly could be confused with vaginal cells. Seldom spermatozoa are found in alcoholic extracts of a stain; they are detected by other methods.<sup>33</sup>

*The Spermiogram and Its Significance in the Identification of Stains.*—Normal semen contains about 20 to 25 per cent of immature, aged and abnormal spermatozoa and only 0.25 to 2 per cent of immature cells of the spermatogenesis in relation to 100 cellular elements. That one will detect these cells in stains from normal semen is therefore unlikely. In abnormal semen the rate of occurrence of defective spermatozoa and of immature cells may be higher. Many in the group of sexual assailants have abnormal semen. In cases of azoospermia the detection of immature cells may lead to the identification of a stain as of seminal origin and may even be helpful in establishing individual identity (Pollák and Joël, 1938, 1939).

*Biometry and Its Significance in Establishing Individual Identity.*—Mönch claims that the size and the shape of the head of the spermatozoon are constant and that biometric curves of two semen samples may be similar but are not identical. As stated before, there seem to be limitations to the value of biometric curves for individual identification: The number of normally formed spermatozoa changes with repeated ejaculation. After spontaneous emission abnormal forms and especially giant forms of spermatozoa are found in higher numbers than are usually present in the semen of the same man. The morphologic aspects of the spermatozoa change with irregularities of the sexual life, with local abnormalities of the sexual organs, with localized remote or with generalized diseases. In addition, seminal stains are much less suitable for biometric studies than fresh specimens. Owing to the adhesion of the spermatozoa to the fibers of the

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33. 196, 197, 293.



cloth, one sees but few spermatozoa in their full outline, whatever method is used for demonstrating the spermatozoa.<sup>34</sup>

*Morphologic Differentiation Between Human and Animal Semen.*—The change of the structure of spermatozoa may proceed so far that the cytologic distinction between human and animal spermatozoa appears difficult. The characteristics of the spermatozoa of various species are well known, and usually the distinction is an easy one. The possibility of confusion is denied by most investigators, but admitted by others. Cases of bestiality or sodomy are often mentioned in the medicolegal literature. Today they are rare. The question may be raised seldom but should be kept in mind. It is answered by immunologic methods.<sup>35</sup>

## RECOVERY OF SPERMATOOZOA FROM DRIED STAINS

### MATERIAL FOR EXAMINATION; HISTORY AND SURVEY OF VARIOUS METHODS

The search for stains should be thorough because the choice of material for examination is decisive in respect to the results of microscopic examination. Stains on nonabsorbent materials are more readily examined than stains on absorbent fabric. The central portion of the stain usually contains the largest number of spermatozoa, owing to the slower drying of the center.<sup>36</sup>

The examination can be conducted in different ways: If the stain is superficial and scaly, material can be scraped away, and being comparatively free from fibrillar material, it allows the cellular elements to be readily observed. Stains on absorbent material are being examined without or with destruction of the supporting material.

Soaking the cloth and searching for the spermatozoa in the extract was the first method applied (1837). Later, spermatozoa separated from the mechanically (1860) or chemically (1882) destroyed support have been recovered. Other methods include demonstration of spermatozoa in situ after more (1857) or less (1864) complete destruction or with preservation (1928) of the stained material.

### DEMONSTRATION OF SPERMATOOZOA IN SITU WITHOUT DESTRUCTION OF THE SUPPORT

The simplest way of demonstrating spermatozoa in their original position in a stain seems to be to color the whole material. Cloth stained with diluted Giemsa's solution after fixation of the stain in alcohol has been observed by special illumination. As an alternative method, May-Grünwald, Jenner's, Ehrlich's or Pappenheim's stain has

34. 203 a and c, 337 a and b.

35. 10, 87 a and d, 138 b and d, 331 b.

36. 87 a and d, 114 a, 124, 200 b.

been used. Coloring the material with a phenolic solution of rose bengal and observation in Auer's light have been recommended. Material also has been treated with Pulgher's solution of tannin and crystal violet, a method previously used in demonstrating bacterial flagella.<sup>37</sup>

The material may be silvered instead of colored. Hortega's solution of silver nitrate in sodium carbonate and ammonia or Bielschowsky's solution of silver nitrate in sodium hydroxide and ammonia have been used for impregnation.<sup>38</sup>

#### DEMONSTRATION OF SPERMATOOZOA IN SITU AFTER PARTIAL DESTRUCTION OF THE SUPPORT

*Fibrillation.*—The material may be partially disintegrated by soaking and teasing. The soaking is intended to soften the material and loosen the smaller fibrils and some of the spermatozoa. Iodine and potassium iodide were applied by many investigators for coloring. Others used alcoholic eosin, watery eosin, croceine or acid fuchsin for staining.<sup>39</sup>

Combined stains which color differently the various parts of the spermatozoa were introduced later. Erythrosin in methyl alcohol was used as a direct stain and methylene blue as a counterstain for material furnished by fibrillation. Acid fuchsin and an acid solution of methylene blue formed another combination. Gram's stain was used on top of fuchsin-methylene blue. Soaking the stained cloth in hydrogen peroxide presents another modification. Some workers prefer the simple stain, others are partial to combined ones, and still others submit all these methods to criticism.<sup>40</sup>

Müller's potassium bichromate solution, Kleinenberg's solution of trinitrophenol in sulfuric acid or Mayer's solution of trinitrophenol in nitric acid have been used for fixation. Ammonia has been applied to prevent the coloring of the support. Ammoniacal erythrosin combined with methylene blue or without it, erythrosin dissolved in potassium hydroxide and Giemsa or May-Grünwald stain have been used for coloring after fixation of the material.<sup>41</sup>

#### DEMONSTRATION OF SPERMATOOZOA IN SITU AFTER COMPLETE DESTRUCTION OF THE SUPPORT

*Teasing.*—Mezger was the first to tease fibers from the center of a stain. He moistened the fibers in ammonia water, converted them into fine shreds and observed the disintegrated material microscopically.

37. 66 g<sup>1</sup> and j,<sup>1</sup> 179, 251 a and b.

38. 95, 229, 324.

39. 13 c, 49, 87 a and d, 108 d, 111, 154 b, 161, 170 a and b, 189 a and b, 191 b, 263 a, b and c.

40. 9, 7 j, 26, 96 a, 102, 126, 149, 168 c, 207, 211 a, 212 c, 250, 253, 291 c, 293, 328.

41. 59 a and b, 66 c,<sup>1</sup> 69 a, b and c, 167, 255.

Colored slides are easier to search than uncolored material. Water, ammonia water, saline solution, solution of potassium hydroxide, ammoniacal eosin, glycerin or glycerin-pepsin are used for softening the material. For staining the shreds Roussin's iodine solution, ammoniacal carmine or a combination of both dyes has been applied. Others have used ammoniacal erythrosin alone or combined with Weigert's iron-hematoxylin, acid fuchsin and methylene blue, or iron-hematoxylin and trinitrophenol.<sup>42</sup>

For fixation of teased material Schweizer's copper hydroxide reagent or Müller's solution has been recommended. Ruthenium red, ammoniacal erythrosin, eosin and carmine have been used as stains for fixed material.<sup>43</sup>

Silvering may replace the staining of shreds.<sup>44</sup>

#### DEMONSTRATION OF SPERMATOOZOA SEPARATED FROM THE PRESERVED SUPPORT

*Soaking.*—In spite of some initial opposition, the methods of maceration have been practiced for a long time and are often used even today. Tap water and distilled water are most often used for soaking the material. Some reject distilled water as causing swelling of the heads of the spermatozoa. To prevent evaporation, a covered watch glass should be used. Acetic acid, hydrochloric acid, nitric acid, potassium hydroxide, solutions of sodium carbonate or of mercury bichloride and cold and hot ammonia water are used in a varying concentration and for a varying length of time. Saline solution, glycerin, alcohol, a mixture of acetone, ether, chloroform and purified petroleum benzene (petroleum ether), Paccini's mixture of glycerin, sodium chloride and mercury bichloride or a mixture of glycerin, pepsin and hydrochloric acid are also recommended for soaking the cloth.<sup>45</sup>

Dark field illumination and also polarized light have been used to demonstrate spermatozoa in watery extracts of stains.<sup>46</sup>

The staining of the extracted material may facilitate the detection of cellular elements and their identification as spermatozoa. Ammoniacal eosin, watery eosin, glycerinic eosin solution, ammoniacal or phenolic erythrosin, watery erythrosin, phenolic gentian violet, phenolic

42. 21 *a* and *b*, 66 *i*, *j* and *k*, 80 *a* and *b*, 117, 118, 125, 137 *a*, 176 *a* and *b*, 199, 260, 319.

43. 59 *a* and *b*, 66 *t*, *a*<sup>1</sup> and *b*<sup>1</sup>, 73, 290 *d* and *e*.

44. 102, 168 *c* and *d*.

45. 12, 13 *b*, *c* and *d*, 17, 37, 46 *b*, 47, 53, 65, 74 *b* and *c*, 86 *a* and *b*, 87 *a*, *b*, *d* and *e*, 88, 95, 99, 105, 107, 109, 117, 124, 148, 158, 159, 189 *c*, 191 *a*, 195, 205, 207, 212 *c*, *f* and *g*, 228, 236, 251 *c*, 252, 256, 285 *a*, 292, 303.

46. 214 *a* and *b*, 234.

fuchsin, watery crocein, watery neutral safranin or alkaline methylene blue is used for this purpose. Maceration of the stained cloth in a solution of mercury bichloride and staining of the extract with methylene blue or with hematoxylin supposedly reveal two hematoïd granules in the head of the spermatozoon. These granules have been proved to be artefacts.<sup>47</sup>

As to combined stains, dried extracts have been colored with alum-carmine, differentiated with acetic acid and counterstained with malachite green. Methylene blue or hematoxylin and eosin and also the May-Grünwald stain have been applied to color the various parts of the spermatozoa differently.<sup>48</sup>

*Soaking and Filtration.*—Repeated maceration in water and ammonia water or alcohol and filtration of the extracts have been recommended to concentrate the material furnished by extraction (Bayard, 1839, 1844, 1846).

*Soaking and Squeezing.*—Maceration in water or saline solution and subsequent squeezing of the cloth have been introduced as another improvement for the examination of unstained extracted material.<sup>49</sup>

Soaking in dilute hydrochloric acid, squeezing and staining with Friedländer's or Böhmer's alum-hematoxylin and with eosin, or vice versa, or with Grenacher's alum-carmine and with eosin, or with Bismarck brown Y and eosin were combined in some prescriptions. Acidified water with methyl green also was recommended. These methods have been often modified, recommended by some workers and rejected by others.<sup>50</sup>

A thin layer of acacia may be applied on the moistened cloth, the preparation pressed against slides, and the adherent material stained with Baccchi's acid fuchsin and methylene blue (Gabbi, 1914 *b*, 1915).

*Soaking and Centrifugation.*—Another way to concentrate the watery or saline extract of a stain is that of centrifuging it. The sediment may be colored with tincture of iodine and alcoholic Biebrich scarlet, water soluble. Centrifugation may be preceded by soaking the cloth in water with thymol, in a solution of mercury bichloride, in Schweizer's reagent modified, in dilute antiformin or in ether. For staining, ammoniacal erythrosin, watery eosin, Ziehl's carbolfuchsin diluted, acid fuchsin-methylene blue, gentian violet or india ink is used with variable success.<sup>51</sup>

47. 7 *d*, 62, 87 *a*, *b*, *d* and *e*, 95, 105, 158, 326 *a* and *b*, 189 *c*, 196, 228, 236, 256, 265, 284.

48. 32, 207, 282.

49. 2, 148, 271, 305 *a* and *b*.

50. 39, 89 *b*, 178, 184, 213, 286, 318.

51. 58, 99, 149, 228, 242 *e*, *f* and *g*, 244, 317, 330 *a* and *b*.

# DÉMONSTRATION OF SPERMATOOA SEPARATED FROM THE SUPPORT WITH THE LATTER'S PARTIAL DESTRUCTION

*Scraping.*—Often the stained cloth is moistened with water, the fabric then scraped with a knife and the material obtained examined.<sup>52</sup>

Staining of the material furnished by scraping moistened cloth may render the differentiation of the spermatozoa from the fibers of the support easier. Carbolthionine solution, Pulgher's crystal violet, eosin and methyl green, and wool black and diluted Löffler's alkaline methylene blue are being used.<sup>53</sup>

Soaking of the piece of stained fabric in Müller's potassium bichromate solution has been combined with squeezing and scraping of the material onto a slide with a scalpel. The heat-fixed slide is stained with Leishman's stain.

A different method has been recommended: Pieces of cloth are tied into small bundles, stained with ammoniacal eosin, then soaked in ammonia water, placed on slides and covered with a mixture of acacia, sugar and albumin; finally the hardened layers are separated with a knife except for the last one.<sup>54</sup>

## DEMONSTRATION OF SPERMATOOA SEPARATED FROM THE COMPLETELY DESTROYED SUPPORT

*Decomposition.*—Mechanically, only partial destruction of the stained cloth may be achieved. Chemically, complete destruction is obtainable. The resistance of the spermatozoa to chemicals is the basis for these methods. Diluted sulfuric acid has been used by some to destroy all organic matter except the spermatozoa. Others have recommended sulfuric acid and distilled water for the destruction of the support, subsequent washing of the material and staining with alcoholic eosin. Iodine or iron-hematoxylin with trinitrophenol and iodine and Van Gieson's stain have been applied for material destroyed in dilute acid. Concentrated sulfuric acid was introduced first by Grigoryeff. The destructiveness of the acid was combined with the ability of potassium cyanide to dissolve protein matter and with the protective qualities of tannin. The use of sulfuric acid and tannin and coloring with alcoholic phenolic erythrosin and alcoholic malachite green represent a further modification of the acid destruction method. The acid is replaced by Schweizer's copper hydroxide reagent and the material stained with erythrosin.<sup>55</sup>

52. 7 *a*, *b*, *c* and *d*, 33, 48, 154 *a* and *b*, 160, 166, 167, 261, 322 *a* and *c*.

53. 179, 183, 325 *c* and *d*, 337 *a* and *b*.

54. 1, 2, 4, 5, 6, 7 *a*, *b*, *c* and *d*, 168 *b* and *r*, 225 *f*, *g* and *h*, 299, 338.

55. 7 *d*, 66 *d*, 66 *d*,<sup>1</sup> 98, 101, 108 *d*, 119 *a* and *b*, 137 *a*, 168 *i* and *j*, 188, 207, 272, 326 *a*, 328, 332.

*Carbonization.*—The upper limit of destruction may be reached by placing the cloth in a crucible with concentrated sulfuric acid and heating the crucible over a flame. This procedure has provoked criticism.<sup>56</sup>

#### COMMENT ON THE METHODS OF MICROSCOPIC EXAMINATION OF DRIED STAINS

*Démonstration of Spermatozoa in Situ.*—The demonstration of spermatozoa in situ without destruction of the support is difficult. Only very thin cloth can be successfully subjected to this method of study. Among the various stains and impregnation methods recommended for

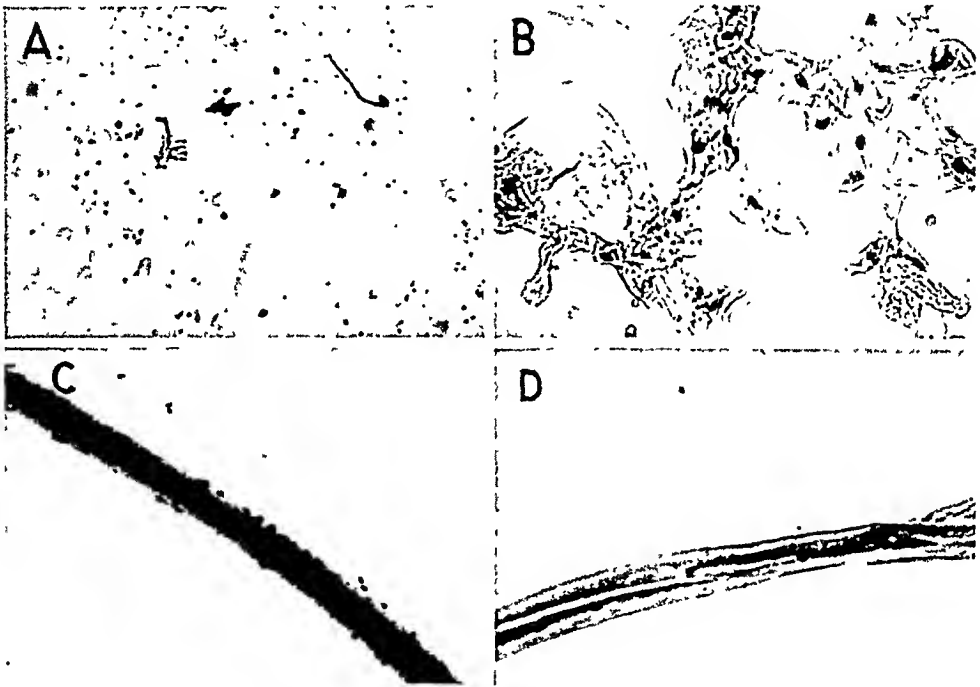


Fig. 2.—A comparison between the results of maceration and the results of dissociation of the support. The result of soaking in a 0.9 per cent solution of sodium chloride for twenty-four hours is shown in *A*, a seminal stain, and *B*, a vaginal stain. The result of teasing of fibers into finest shreds is shown in *C*, a seminal stain, and *D*, a vaginal stain.  $\times 300$ .

this purpose the most preferable one is a combination of the Jenner, May-Grünwald and Giemsa stains. Most of the dyes and the silver solutions stain or impregnate the supporting material as well as the spermatozoa. Generally, these methods cannot be recommended.

The partial mechanical destruction of the support renders the demonstration of spermatozoa in situ easier. Fixation of the material is unnecessary, because the spermatozoa adhere to the fibers. Many

56. 66 c,<sup>1</sup> 86 c.

modifications of the fibrillation method have been described, in which various dyes have been used. The claims concerning the specificity of stains for spermatozoa cannot be substantiated. No method in this group deserves consideration.

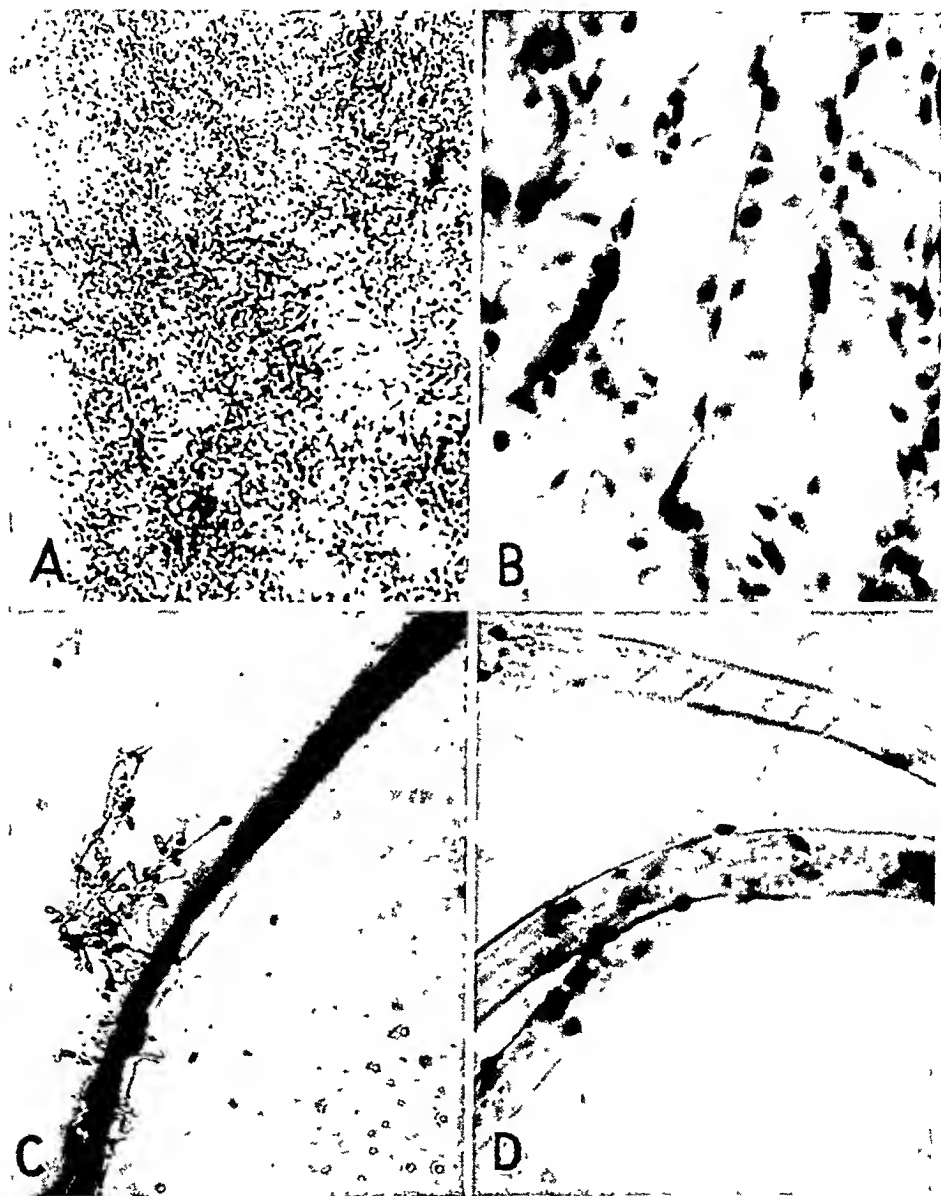


Fig. 3.—Methods suitable for the recovery of spermatozoa from stains. *A* and *B* represent chemical destruction of the support. *A* demonstrates that after twelve hours in concentrated sulfuric acid the number of spermatozoa is as high as that of a fresh sample (F 120). *B* shows the details visible after chemical decomposition ( $\times 400$ ). *C* and *D* represent mechanical destruction. *C* shows the spermatozoa still adherent to the support after twenty minutes' boiling in tap water ( $\times 360$ ). *D* shows the details visible after mechanical dissociation ( $\times 400$ ).

The further destruction of the support by teasing the fibers into the finest shreds furnishes the desired results. Again, fixation of the material can be omitted; stiff material may have to be softened. Among all the solutions recommended, tap water, ethyl alcohol or methyl alcohol serves best, provided that the dry material cannot be teased. Among the stains, erythrosin combined with iron-hematoxylin, as recommended by Ellermann (1911) furnishes the best results, and his method can be accepted as the best one for the demonstration of spermatozoa in situ.

*Isolation of Spermatozoa.*—The demonstration of spermatozoa separated from the preserved support is the oldest and most commonly used method. In most instances it is unsuccessful because of the adherence of the spermatozoa to the support. Among the numerous reagents recommended for soaking stained material dilute hydrochloric acid is by far the best. Water with thymol furnishes some results. Staining of extracts is helpful in the detection of the cellular elements. Filtration, squeezing and centrifugation of the extracts may concentrate the material but damage the spermatozoa to such an extent that the identification of the organisms is unreliable. Maceration, whether applied alone or in combination with other procedures, cannot be recommended. Its value is in demonstrating cellular elements other than spermatozoa.

Scraping of moistened cloth and also embedding and scraping furnish hardly a preserved spermatozoon.

The complete decomposition of the support by chemical means is the only way toward satisfactory results. Among the reagents applied, concentrated sulfuric acid, as recommended by Grigoryeff (1902 *d*), furnishes the best results when one attempts to demonstrate spermatozoa separated from the stained material.

#### ATTEMPTS TO IMPROVE THE MORE VALUABLE METHODS

The large number and the variety of procedures recommended for the detection of spermatozoa in seminal stains are rather confusing. In textbooks the chapters concerning the examination of seminal stains are usually meager. Only a few reviews concerning this matter have been published, the last one in 1914. A voluminous review has been published in the Spanish language, but the writer chose as the best methods unsuitable ones, such as embedding and scraping. Less extensive reviews have been published in Germany, but the authors of these failed to pay any attention to papers other than those published or abstracted in the German language.<sup>57</sup>

In order to evaluate all the methods and to judge the competitive procedures fairly, it is necessary to try them out. I have undertaken

57. 86 *b*, 142, 168 *c*, 249.



✓ this task. Two methods are found to furnish satisfactory results: (1) the teasing of fibers of cloth into the finest possible shreds followed by staining with ammoniacal erythrosin and iron-hematoxylin; (2) the dissociation of the material in cold or slightly warmed concentrated sulfuric acid.

The two methods which will be described in detail at the end of this review have been subjected to experimentation in order to simplify the technic or to improve the results. The mechanical destruction of the fabric is simple. Dry material or material first moistened with a drop of water, ethyl alcohol or methyl alcohol is teased with two needles. Instead of erythrosin, eosin may be used, and instead of iron-hematoxylin, hematoxylin. Such a change is of no advantage, as the time of staining must be prolonged in such a case. The chemical decomposition of the material is equally simple. The only modification to be recommended is the shortening of the time of digestion, which may be accomplished by placing the watch glass with the substrate in the acid into an incubator at about 50 C. The most suitable moment for the microscopic examination of the material has arrived when only a skeleton of the stained matter flows in the acid. This skeleton is taken out, transferred to a slide, covered with a cover glass and observed under low and high power lenses. The preservation of such a slide is difficult. However, after six weeks some of the spermatozoa are still unchanged. Repeated lavage of the slides in water fails to change the results. Neutralization of the acid with sodium bicarbonate or sodium hydroxide results in complete destruction of the cells. Attempts to imitate the procedures used in the histologic technic for decalcification meet with failure; sodium sulfate, lithium sulfate or potassium alum applied in 5 per cent watery solutions for one or two days with one change and washing in distilled water result in disintegration of the spermatozoa. Staining of the slides furnished by chemical destruction is impossible because of the decomposition of dyes through the action of the acid, but is unnecessary, as the spermatozoa appear dark brown or black.

In addition to the methods recommended in the literature, the best procedures used for microscopic studies of spermatozoa and cells in fresh seminal smears have been tried out for comparative purposes. After heat fixation, slides were treated with diluted solution of sodium hypochlorite U. S. P. (Dakin's solution), stained with a mixture of carbolfuchsin and alcoholic eosin and counterstained with watery methylene blue. After fixation in Schaudinn's fluid, slides were stained with watery eosin and hematoxylin. After fixation in alcohol, slides were stained with hemalum and eosin, with the Ehrlich-Biondi-

Heidenhain polychrome stain, which is a mixture of acid fuchsin, orange G and methyl green, and with Pappenheim's methyl green-pyronine. Giemsa's azure-eosin was applied after methyl alcohol fixation of shreds. None of these methods gives exceptionally good results with dried material. For the study of cellular structures, the last method may have some advantage against the quicker erythrosin and iron-hematoxylin stain.<sup>58</sup>

Finally, an attempt was made by me to find a new way of demonstrating cellular elements in stains. Pieces of linen, half-linen, silk, rayon, wool, cotton and fur of different qualities and colors were handled similarly to tissues. Paraffin sections of varying thickness were stained with all common methods. Owing to the nature of the material, only a few spermatozoa are seen in their full outline. Boiling of the cloth for ten minutes in water before dehydration and embedding facilitates the detection of spermatozoa, as they separate from the shreds. Besides spermatozoa, other cellular elements of semen are seen. The picture generally is much the same as that of the slides prepared by teasing fibers into fine shreds. The proper thickness of sections was found to be between 4 and 6 microns. The erythrosin and iron-hematoxylin method seems to be the best for these sections.

The study of the morphologic characteristics of spermatozoa in dried material is difficult. Few spermatozoa can be seen in their full outline. Most of them are not seen from above, but tangentially on the edge of shreds, where they lie attached to the material. The tails are twisted around the finest hairs, the neck adheres, and the head often forms an angle with the axis of the shred. Only spermatozoa lying near the shreds or on the shreds appear plainly.

The reasons for the failure of some and the success of other methods are obvious. The separation of the spermatozoa from the support is seldom successful; the destruction of the soiled material is the basic requirement for the demonstration of spermatozoa in dried material. A relation between the age of the stain and the time necessary to free the spermatozoa cannot be found. Many negative results and the difficulties mentioned by various authors are due to the choice of unsuitable methods. A complaint of difficulty in detecting so much as a single spermatozoon in a seminal stain produced by normal semen containing millions of spermatozoa is almost invariably due to attempts to demonstrate the spermatozoa separated from the cloth. The adherence of the spermatozoa can be demonstrated by experiments involving the artificial removal of seminal stains.

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58. 45, 127, 203 *a* and *b*, 239 *b* and *c*, 266.

## ARTIFICIAL REMOVAL OF STAINS

*Boiling Water.*—The various authors reached contradictory conclusions regarding the artificial removal of seminal stains. Some did not find any spermatozoa after boiling the stained cloth in water; others found spermatozoa after five, ten or twenty minutes' boiling.<sup>59</sup>

In my experiments the spermatozoa first disappeared from the outline of the shreds: after the material had been boiled for twenty or thirty minutes in tap water, the spermatozoa left the fibers in groups. At this point the examination of the water was successful. After as short a time as fifteen minutes of boiling, the heads of the spermatozoa were swollen and deformed. Simultaneous use of the method of teasing and the method of chemical destruction showed the first procedure more effective, as the spermatozoa adhered to the shreds and were increasingly deformed with the more advanced dissociation of the acid. After one

TABLE 1.—*Spermatozoal Findings in Boiled Cloth*

Minutes	Teasing of Cloth	Acid Destruction	Washings
5	+++ normal findings	+++ normal findings	0 none found
10	+++ normal findings	+++ normal findings	± isolated heads
15	++ lower number	++ lower number	+ same, tails +
20	++ same, swollen	+/- few found	++ some, tails ++
25	++ same, swollen	±/0 few or none found	+++ deformed spermatozoa
30	+ harder to find	0 none found	+ same, fewer
35	±/0 very hard to find	0 none found	± same, fewer
40	±/0 very hard to find	0 none found	± same, fewer
45	±/0 very hard to find	0 none found	± same, fewer
50	±/0 few, identity ?	0 none found	0 none found
55	±/0 few, identity ??	0 none found	0 none found
60	±/0 few, identity ??	0 none found	0 none found

hour, the spermatozoa were still visible on shreds of the cloth, but no one could identify them as such in unknown material. The results of experiments with different types of fabric were approximately the same.

The routine which I have observed is as follows: Pieces of cloth are boiled in tap water, samples are cut every five minutes, and the water is changed twice before boiling for the next period of five minutes. The results are shown in table 1.

*Soapy Water.*—Boiling the material in ordinary water will remove a seminal stain, but only very slowly. Boiling in soapy water interferes with results within five minutes, although this has been denied by some investigators.<sup>60</sup>

Comparative experiments with a 3 per cent solution of soap flakes (Rinso) and a 10 per cent solution of sodium carbonate reveal that after boiling for five minutes only a few isolated heads may be found on the fibers of the cloth. The chemical destruction furnishes some

59. 89 b, 212 c, f and g, 291 d, 332.

60. 57, 291 d, 332.

spermatozoa with shrunken heads and a somewhat greater number of isolated tails. In the soapy water or the sodium carbonate solution no cells can be detected. The destruction of the spermatozoa in hot alkaline solutions proceeds so far within five minutes that their identification becomes practically impossible.

*Rubbing in Cold Water, Soapy Water, Alcohol.*—Pieces of stained material may be washed with cold water and mechanically rubbed until the stain disappears. This takes only a few minutes. As long as the stain is not removed completely, its outline being grossly still visible, the results of the microscopic examination are the same as before. With complete removal of the stain, the results are changed, and the output is lower, but a sufficient number of spermatozoa for reliable diagnosis can usually be detected.

The stain disappears quickly if the stained material is rubbed with a piece of linen moistened in soapy water. After a minute's washing the detection of spermatozoa is almost impossible. One sees only a few deformed spermatozoa on the shreds, and in some samples, none. In slides made by the method of chemical destruction one sees much debris, a few separated heads with necks, but no complete spermatozoa. The identification of elements as spermatozoa is practically impossible.

As long as a seminal stain is grossly visible or can be detected on the support, the result of microscopic examination does not differ from that under normal conditions. If the material is washed with plain water or boiled in tap water, the detection of the spermatozoa may be possible although macroscopically the stain has disappeared. In practice, this has not too much importance because probably no one would think to examine the material for spermatozoa unless he first detected a stain. Alcohol is not suitable for the removal of stains. A solution of soap or sodium carbonate renders the detection of spermatozoa impossible, whether applied cold or hot.

Questions regarding natural changes in stains cannot be answered by laboratory experiments. It is hardly possible to imitate in the laboratory the change of temperature and humidity which, according to some authors, explains many negative findings in the tropics. The method applied is often responsible for negative results. Putrefaction, also, might be expected to interfere with the examination, but laboratory experiments with artificially infected semen and seminal stains fail to confirm this supposition. There has been a tendency to overrate the influence of external factors on the results of the examination of seminal stains. If spermatozoa cannot be detected by dissociation, destruction and biopsy after boiling of the material, one immediately must ask whether semen is present or whether the stain conceivably originates from semen without spermatozoa. These questions may be answered by other methods than the microscopic examination of the material.

COMMENTS ON THE MICROCHEMICAL TESTS FOR SEMEN  
AND SEMINAL STAINS

*Tests for Choline.*—Because of the high content of choline group substances in human semen, the reagents for choline frequently give a

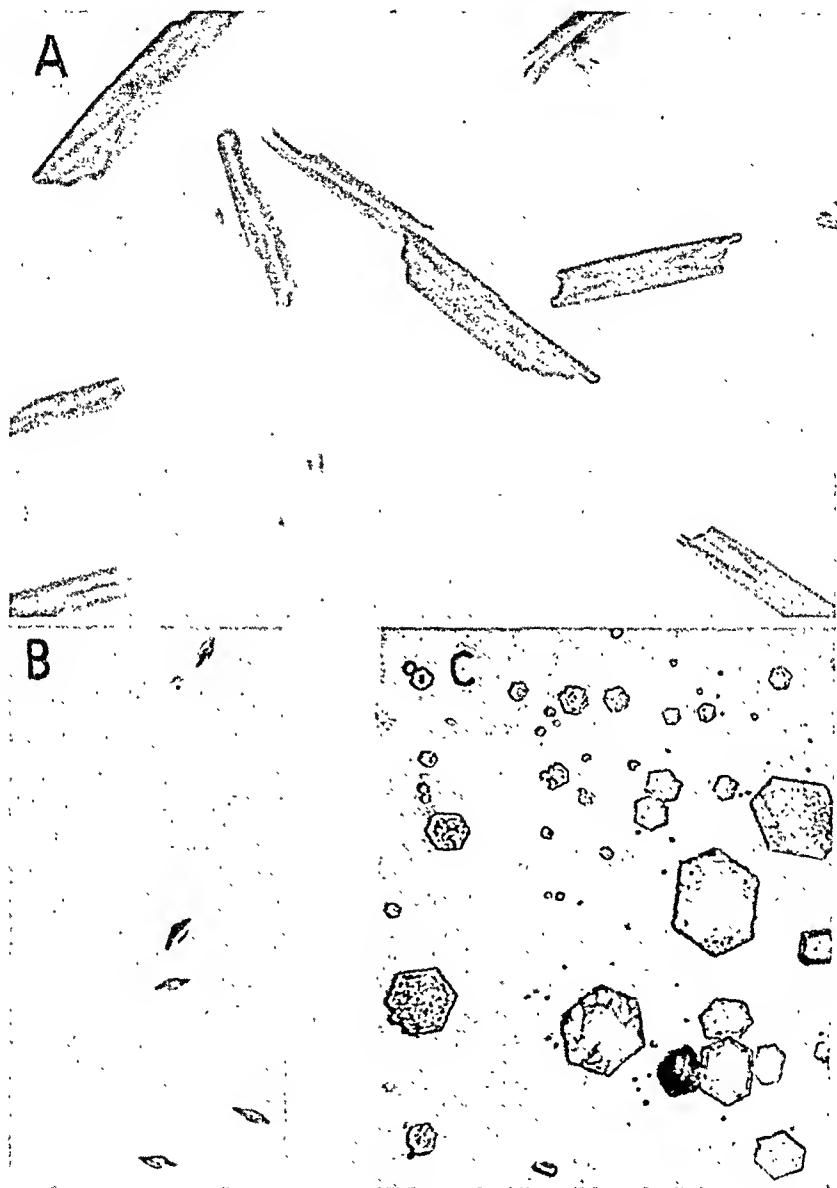


Fig. 4.—Crystals resulting from microchemical reactions with semen ( $\times 810$ ):  
A, Florence's reaction; B, Barberio's reaction; C, Lecha-Marzo's reaction.

positive reaction. The chemical properties of the reacting substances explain many failures experienced with the tests. Exposure to water, changes of putrefaction or admixture of protein may account for many negative results. The presence or absence of spermatozoa is immaterial.

The tests are not specific, because of the presence of reacting substances in material other than human semen.

The original Florence test is better than the various modifications, which only change the amount, distribution, size and form of the resulting crystals. The formation of crystals does not depend on the reaction of the medium but on the concentration of choline. After

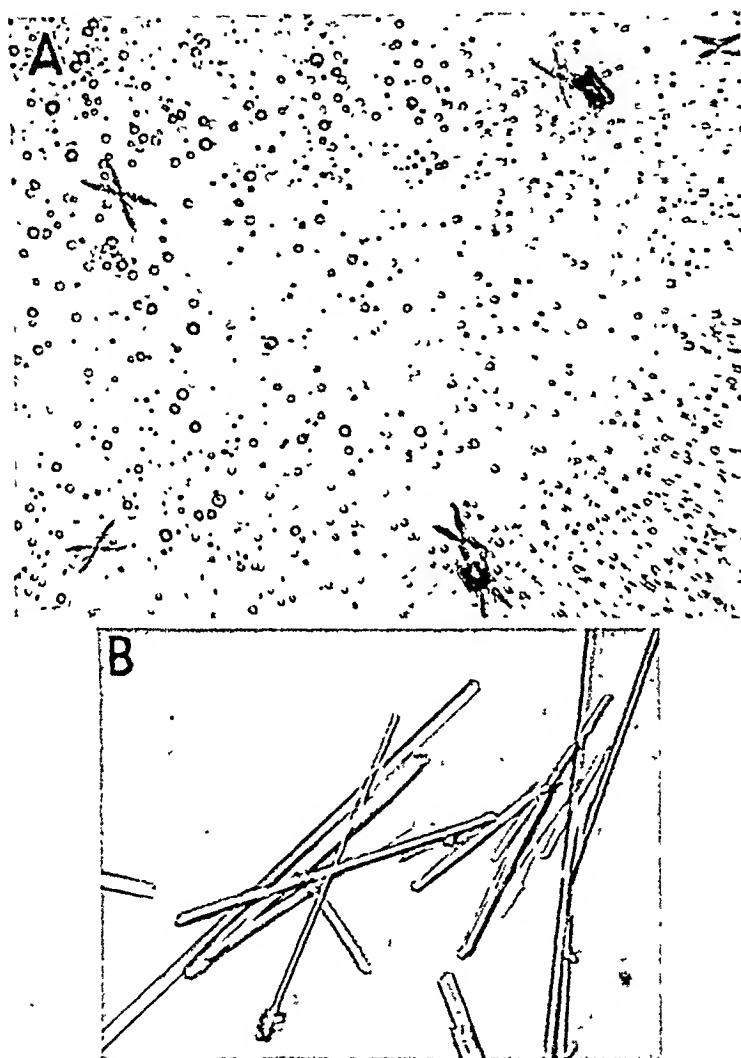


Fig. 5.—Crystals resulting from microchemical reactions with semen ( $\times 810$ ): A, de Dominici's reaction; B, Niederland's reaction.

maceration of the stained material with phosphoric acid, the typical crystals appear surrounded by an amorphous precipitate which may correspond to acetylcholine (Booth).

*Tests for Spermine.*—As in the first group of reactions, the reacting substance explains the results achieved. Spermine is less sensitive to humidity, putrefaction and admixtures of protein matter than choline.

In spite of that, the sensitivity of all tests for spermine devised is lower than that of the reagents for choline. The roles of the spermatozoa and the prostatic secretion are the same as in the first group of tests. Spermine and spermine phosphate are present in animal semen as well as in human semen, and also in other human, animal and vegetable material. As with the tests for choline, so with spermine positive results do not indicate human semen nor do negative results exclude it.

The Barberio test is the best known among the tests for spermine. The only modification improving the results is the one based on concentration of the material by evaporation (De Dominicis, 1907 *e*; 1908 *g*).

In the combination of the Florence and Barberio reactions, the higher sensitivity of the test for choline becomes evident. Usually, only Florence's crystals are present. Bocarius' test with phosphotungstic acid is less sensitive than Barberio's reaction; the Lecha-Marzo phosphomolybdic acid test has the advantage that large crystals are formed. Among all the crystals described with this reagent only the yellowish hexagonal plates are characteristic. Puranen's naphthol yellow S test and modifications of it are inferior to Barberio's test, the sensitivity of those tests being far below the original claims.<sup>120</sup>

*Tests for Choline and Spermine Combined.*—A reaction combining the two principles should have all the advantages and disadvantages of other tests. In practice, the reagents combine readily with spermine, but their sensitivity for choline is much lower. De Dominicis' test furnishes about nine times more yellow needles than red squares. With concentration of the material the original relation of 9:1 may be changed to one as low as 7:3, owing to the increase of choline compound crystals. Various modifications change merely the appearance of the crystals (De Dominicis, 1912 *v*, *w* and *y*).

*Test for Calcium.*—The high content of calcium in human semen renders the test positive. Addition of water, putrefaction and admixture of protein do not make the results negative. Owing to the presence of calcium in almost any biologic material, there is no organ or species specificity. The uselessness of Niederland's test is obvious.

#### COMPARISON OF ALL MICROCHEMICAL REACTIONS FOR SEMEN

Only a few investigators have compared the several microreactions for semen. Florence's test and De Dominicis' may be better than all the other reactions.

120. 25 *g*, 168 *k*, *l*, *m*, *n* and *o*, 247 *a* and *b*.

A titer of 1:500 is asked as the measure of the minimum sensitivity for a suitable microreaction for semen. Only Florence's test is apt to fulfill this demand. A negative result of the Florence test and a positive result of the De Dominicis test, the latter used as a control of spermine, seem to be more valuable than the results of the other tests.<sup>122</sup>

None of all the tests introduced either indicates or excludes human semen. All the reagents used react with various substances, especially with many alkaloids. In addition, choline, spermine and calcium all have a universal distribution. None of the tests is sensitive and specific enough to identify material as semen. Differentiation between human and animal matter is impossible, and so is the distinction between semen and other material.

The chemical composition of human semen gives little hope that a suitable test will be detected. Most of the chemical constituents which are present in a high amount have been already used as the basis for microreactions. Phosphorus is one of the few substances left for further experimentation. A reaction for phosphorus would be very sensitive, but not specific. Its value would be the same as that of the test for calcium.

Other methods, based on a different principle, have to replace the microchemical tests in order that one may answer the questions which cannot be solved by morphologic examination of semen stains.

#### IMMUNOLOGIC CHARACTER OF SEMEN AND SEMINAL STAINS

The differentiation between human and animal material is a comparatively easy procedure. With sufficient controls the results are reliable. Antisemen serums may be used instead of antiblood serums. They answer two questions at the same time. An organ-specific precipitin serum which reacts with any semen, regardless of the species, can be produced. Its practical value is low because of the low titer for heterologous material. The titer of such serums is higher with fresh semen than with extracts of dried seminal stains.

An anti-human-semen precipitin serum of sufficiently high titer is most suitable for use in tests to solve the question of origin of the material. The absorption technic as recommended by Hektoen and Rukstinat is the basis of the practical identification of semen and seminal stains. Serums from rabbits immunized with fresh or dried semen or with fresh or dried spermatozoa-free prostatic secretions from men and animals will give cross reactions. Fresh material produces serum of a higher titer than does an extract of a dried stain (Hektoen, 1922, 1928).

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122. 235, 324.



Admixture of other biologic material does not interfere with the results of precipitin tests provided that these be done after absorption. Sometimes, however, the titer of an absorption-treated serum for semen is too low to permit conclusions. Moisture and putrefaction disturb the results of precipitin tests, usually in the sense of rendering them positive. Artificial removal of stains disturbs the reactions. Dilution of the material or washing may render the results negative, while alcohol, soap and sodium bicarbonate cause positive results. The precipitation seen with extracts of unstained parts of the cloth is due to traces of soap or bleaching powder. In doing tests, controls employing unstained material are indispensable (Hektoen, 1922).

Immunologic individualization on the basis of precipitin tests using antiserums produced by injection of motile spermatozoa has rather theoretic value. In practice it may be difficult to get the necessary material repeatedly. The long time required for immunization is one disadvantage, and the necessity for the cooperation of the suspected person another.

Grouping is more suitable and may be performed without the cooperation of the suspected persons. The technic has to be carefully chosen. Extraction of seminal stains in concentrated A and B serum of a known titer simplifies the procedure. The absorption is accomplished within two hours at 37 C. and only for convenience is this period prolonged to two hours at room temperature and over night in the refrigerator. The erythrocytes may be used in a 0.25 per cent suspension. Equal measured parts of the diluted serum used for absorption and of the cell suspension may be used instead of drops. The agglutination may be read grossly after centrifuging the material or microscopically in hanging drops after one hour. A combination of the two readings is advisable. The final titer is calculated on the basis of the final dilution of serum of a known strength previously determined under identical conditions.

The disturbing factors in grouping are much the same as in the precipitation tests. The most common factor responsible for interference is absorption by the unstained cloth. Interference may also be caused by factors similar to those responsible for nonspecific results in precipitin tests. As with other immunologic reactions, the use of all possible controls renders the procedure more valuable. The grouping of semen and seminal stains represents an important step in the routine examination of this material and should be carried out regardless of whether spermatozoa are detected or not.

If one maintains all necessary controls, the immunologic procedure may answer very often all the questions which cannot be solved by means of the macroscopic, microscopic and microchemical examination of semen

and seminal stains. The chief need is that one keep constantly aware of all limitations of any method used and that one draw conclusions carefully, uninfluenced by preconceptions. In oral and written statements, the positive as well as the negative findings should be reported with strict impartiality and without comment to the persons entitled to make inquiries about a sexual offense.

### RECOMMENDED ROUTINES

The study of the work of others and my own experiments led to the elaboration of a course of routine examination of semen and seminal stains which differs from the one commonly used.

The analysis of fresh semen is discussed under the head "Technic of Examination of Fresh Fluid Semen," on pages 147 to 152.

### EXAMINATION OF SEMINAL STAINS

*Macroscopic Examination.*—The gross examination of fresh semen concerns the amount, color, odor and consistency. In the case of stains, their detection and localization and the coordination of their position with the circumstances at the scene of the crime are more important than the other macroscopic features. The size and the number of stains may be helpful in differentiating between spontaneous emission and wiping, and their arrangement may tell whether a piece of cloth has been used for cleansing the penis, the vagina or other places. Ultraviolet rays or a 0.01 per cent solution of alizarin sodium sulfate in distilled water applied on the material may facilitate the detection of stains which have not been seen before. These expedients help locate stains but do not prove that the stains are of seminal origin. They do not interfere with further examination of the stains.

*Microscopic Examination.*—The microscopic examination is performed to detect cellular elements characteristic of semen. From fluid material one can make smears and observe them unstained and after coloring, preferably with Giemsa's stain applied in the same way as to blood smears. Spermatozoa and cells of the spermatogenesis are readily detected in fresh material, and spermiograms and biometric curves based on studies of the cellular structures are worked out.

In the case of stains the destruction of the material stained is necessary. Three methods may be applied:

1. A fiber of the center of the suspected stain is teased out into finest shreds, either dry or in a drop of water, alcohol or methyl alcohol. The shreds are transferred to a slide, stained for one minute with a 0.5 per cent solution of erythrosin in ammonia water, blotted between filter paper, washed shortly with tap water, counterstained for two minutes with Weigert's iron-hematoxylin freshly prepared, washed again with tap water, blotted, mounted in balsam and finally

covered with a cover slip. In this way the original position of the spermatozoa on the shreds of the fabric is demonstrated.

For morphologic studies, the shreds may be fixed in a drop of methyl alcohol for three minutes, stained fifteen to thirty minutes with a freshly prepared solution of Giemsa's stain, blotted between filter paper, washed with distilled water, clarified in xylene for five minutes and mounted under a cover slip. Structural details may be seen in the spermatozoa which lie on or beside the shreds.

2. The second method is the chemical decomposition of the material stained. The central part of the suspected stain is placed in concentrated sulfuric acid in a covered watch glass, which is kept in an incubator at 50 C. The material is checked grossly each hour. The moment the material is visible as a skeleton, it is transferred to a slide and overlaid with a cover slip. The time required for digestion depends on the material stained. Textiles may be destroyed within one to four hours. The number of spermatozoa may be demonstrated in this way.

3. As a third method, biopsy of seminal stains may be utilized: Pieces of the material are boiled for fifteen minutes in tap water and then transferred for fifteen minutes to 70, 80, 90, 100 and 100 per cent alcohol, to alcohol-xylene and to xylene. They are kept for thirty minutes in petrolatum and for the same length of time in paraffin; finally they are embedded. Sections 4 to 6 microns in thickness are stained with erythrosin and iron-hematoxylin. The resulting picture is much the same as after teasing. In addition to spermatozoa, the other cellular elements are visible.

Maceration of a piece of cloth in saline solution or tap water for twenty-four hours is useful when the demonstration of cellular elements other than spermatozoa is desired. For the recovery of spermatozoa this method is unsuitable. The fluid may be centrifuged and the sediment examined after staining with Giemsa's and with Gram's stain. Maceration in absolute alcohol in a covered watch glass kept in the ice box over night is necessary if glycogen granules in vaginal epithelium cells are to be shown. Best's carmine is the most suitable stain for this material.

*Immunologic Examination.*—Precipitin tests are performed in any case in which spermatozoa cannot be detected in the material. They also serve in the differentiation between human and animal material. A certain number of seminal stains with and without spermatozoa and also similar samples of the semen of various animals on all kinds of fabric should be kept prepared for use as controls. An antihuman-semen precipitin serum is prepared in rabbits by six intravenous injections of a mixture of at least three different samples of normal semen. The injections are given at the rate of two a week for three weeks. The titer of the prepared serum is tested. If the titer is sufficiently high (precipitation occurring in a dilution of more than 1:20,000 in twenty minutes at 37 C.), the animals are bled six days after the last injection. The final titer in the illustrative sample (table 2) is twice as high as the detected strength, because of the dilution.

Grouping of the material is also performed regardless of the results of the microscopic search for spermatozoa. The titer of group-specific serums used for absorption is established in simultaneously set up experiments. The final titer has to be figured on the basis of the original strength and the final dilution of the group-specific serums.

TABLE 2.—*Immunologic Identification of a Stain as That of Human Semen*

A. : Absorption for 1 hour at 20 C. and for 12 hours at 4 O.								Result
0.1 cc. of normal serum + 0.1 cc. of human blood diluted 1:200.....								0
0.1 cc. of antiserum serum + 0.1 cc. of human blood diluted 1:200.....								+++
B. : Precipitation read after 1 hour at 20 C. (a ring or a precipitate is indicated by +)								
0.1 Cc. of the Treated Serum (Supernatant Fluid of A) Diluted to								} and { 0.1 Cc. of:
1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	
0	0	0	0	0	0	0	0	Extract of blood stain
+++	+++	+++	++	++	+	0	0	Extract of semen (?) stain
++	++	0	0	0	0	0	0	Extract of cloth
+++	+++	+++	+++	+++	++	+	+	Fresh human semen
+++	++	++	+	0	0	0	0	Animal semen

TABLE 3.—*Grouping of a Semen Stain*

A. : Absorption for 2 hours at 20 C. and for 20 hours at 4 C.		B. : Agglutination read grossly after 4 minutes of centrifugation at 2,000 revolutions per minute and resuspension, then microscopically in hanging drops after 40 minutes at 20 C.					
Stained Cloth of:	in { 0.2 Cc. of Typing Serum:	0.1 Cc. of the Treated Serum Diluted to				} & { 0.1 Cc. of a 0.25 % Red blood Cell Suspension:	Result Group
		1:40	1:80	1:160	1:320		
Blood O	A	+++	+++	++	+	B	O
Blood O	B	+++	+++	++	+	A	
Blood A	A	+++	+++	++	+	B	A
Blood A	B	+	0	0	0	A	
Blood B	A	++	+	0	0	B	B
Blood B	B	+++	+++	++	++	A	
Blood AB	A	+	0	0	0	B	AB
Blood AB	B	0	0	0	0	A	
Semen ?	A	+	0	0	0	B	"B"
Semen ?	B	+++	++	+	+	A	
Cloth	A	+++	+++	++	+	B	O
Cloth	B	+++	+++	++	++	A	
	A	+++	+++	+++	++	B	A
	A	0	0	0	0	A	
	B	0	0	0	0	B	B
	B	+++	+++	+++	++	A	

### GENERAL SUMMARY

A wide variety of laboratory tests may be employed successfully for the identification of material of seminal origin. The choice of methods depends on the nature and the condition of the material and on the specific purpose of the investigation.

The various physical, chemical and immunologic tests applicable to the examination of seminal material have been reviewed, and their respective spheres of usefulness have been described.

Microscopic examination of suspect material for the identification of spermatozoa is the most generally useful procedure because it is least likely to be interfered with by extraneous influences. Frequently the success of such an examination depends on the utilization of special procedures by which cellular structures may be freed from their environment. Of equal and in some respects of greater specificity are the various serologic tests. Unfortunately, certain of the immune properties of seminal material deteriorate rapidly and may be destroyed by the chemical qualities of the environments in which stains are likely to be found.

The many macrochemical and microchemical tests that have been recommended through the years are of limited usefulness even under optimal conditions, and they should be employed only with full appreciation of their limitations.

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## Notes and News

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**Awards.**—C.-E. A. Winslow, Anna R. Lauder professor of public health at Yale University, was awarded the Sedgwick Memorial Medal by the American Public Health Association in recognition of his distinguished service in public health.

Thomas Addis, of Leland Stanford Jr. University, has been awarded the Cullen Prize by the Royal College of Physicians, of Edinburgh, in recognition of his work on Bright's disease.

**Appointments.**—A. C. Ivy, professor of physiology at Northwestern University Medical School, has been appointed scientific director of the Naval Medical Research Institute, at the Naval Center, Bethesda, Md.

Eugene L. Opie, emeritus professor of pathology at Cornell University Medical College, has taken over again the direction of the department during the absence of William Dock, who has been commissioned a major in the army.

**Society News.**—The American College of Physicians has canceled its annual meeting, scheduled to be held in Philadelphia, April 13-16, 1943.

The American Society of Clinical Pathologists has decided to hold its next annual meeting in Chicago on June 4, 5 and 6 at the Drake Hotel.

**Awards of the National Science Fund.**—These awards are of a new type in that they will be given to further the scientific work of the recipient, being designed not only to reward those engaged in research for their accomplishments, but also to assist those with exceptional abilities to carry on further research. Two prizes, each of the value of \$2,000, will be presented, one for 1942 and the other for 1943, "for outstanding contributions to our knowledge of factors affecting the growth of animal cells with particular reference to human cancer." Suggestions are invited by the Advisory Committee of the National Science Fund, 515 Madison Avenue, New York.

**Registry of Ovarian Tumors.**—The American Gynecological Society has established the American Registry of Ovarian Tumors under the charge of five gynecologic pathologists, with Emil Novak as chairman.

**Medical Research Fellowship.**—The Mary Putnam Jacobi Fellowship for Medical Research of \$1,000 of the Women's Medical Association invites applications. The secretary of the committee is Dr. Phoebe L. Du Bois, 150 East Seventy-Third Street, New York.

## Obituaries

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LUDWIG ASCHOFF

1866-1942

Ludwig Aschoff, the eminent German pathologist, according to a report in the issue of the *Schweitzer medizinische Wochenschrift* of September 12, 1942 (page 1017), died on June 24, 1942, at Freiburg, Germany, in his seventy-seventh year. Brief notices have appeared in *The Journal of the American Medical Association* and in *Science*. He had retired as emeritus professor in 1936.

Aschoff was born, the son of a well known physician, in Berlin, on Jan. 10, 1866. From Fischer's "Biographisches Lexikon der hervorragenden Aerzte der letzten fünfzig Jahre" (1932) one learns that, following the German custom, he attended several schools—Bonn, Berlin, Strassburg—graduating at Bonn in 1889. From 1891 to 1893 he served as assistant in von Recklinghausen's Institute at Strassburg, and then for ten years, in the Pathological Institute at Göttingen, "habilitating" in pathologic anatomy in 1894. In 1903 he was called to the chair of pathologic anatomy at Marburg; and in 1906 to Freiburg, where his chief work was done until his retirement for age in 1936.

Search, especially in the "Index Medicus and the Quarterly Cumulative Index Medicus," reveals that Aschoff's long list of some two hundred publications, beginning with "Die Pyelonephritis" in 1893, covered most aspects of morbid anatomy and some of natural history, with many brief remarks at society meetings, obituary notices and other items. He was one of the founders of the German Pathological Society.

His most important scientific work was done before World War I. Appendicitis in its various forms first attracted his attention and held it throughout his professional life, his monograph on the subject appearing in 1908. Turning next to the heart, Aschoff and his pupil Tawara in 1904 discovered the "Aschoff bodies" of the myocardium in rheumatic fever. The description of these structures occupied one page in his six page presentation of myocarditis to the German Pathological Society. It is curious that in neither his summary nor the discussion that followed was the importance of the discovery appreciated. The newly rediscovered bundle of His next claimed their attention. Several articles on this subject by Aschoff, published in journals, and a book by Tawara remain in many aspects the final word on the matter. The terms "thrombosis," "arteriosclerosis," "cholecystitis" and "cholelithiasis" appear frequently in the

titles of his publications. One of the most important of his contributions was the synthesis, based on studies by Kiyono and others, of the now well known reticuloendothelial system. In spite of some continued adverse theoretic criticism, this concept has proved to be of very considerable scientific and practical value. His account of this system is found best in the "Lectures in Pathology" (Hoeber), which he delivered



*Yours faithfully - The list*  
*Ludwig Aschoff*

Autographed photograph of Aschoff (taken about 1927).

in this country in 1924. Aschoff's name is said to be connected with the paradidymis and the paro-ophoron, but I have not been able to trace this connection.

The marked drop in his annual output during the war and for several years thereafter and the quality of this output since that time are mute witnesses to the ravages that have mutilated German medical science in the past thirty years. His last listed publications of any consequence were a series of articles on the normal and the pathologic anatomy of old age (1937) and two on arteriosclerosis in 1939 and 1940.

To many Aschoff is best known for his two volume textbook of pathologic anatomy, which first appeared in 1909 and reached its eighth edition in 1936. Edited by Aschoff, this work compiled by fourteen collaborators was divided into twenty-two parts, of which six were written by Aschoff himself (circulatory disturbances, local defense reactions, the heart and pericardium, the urinary apparatus, the female reproductive apparatus, the digestive organs).

Aschoff's interest in the history of medicine is indicated by occasional articles, such as his Finlayson Lecture, the "History of the Circulation," and by his "Kurze Uebersichtstabelle zur Geschichte der Medizin," first arranged as a supplement to Schwalbe's lectures (1909); this reached a third edition (sixty-one pages), prepared in collaboration with Diepgen, in 1936. As might be expected, the content matter greatly overemphasized Germany's role.

He also edited "Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie" and volume 8 ("Pathologische Anatomie") of the "Handbuch der aerztlichen Erfahrungen im Weltkriege 1914-1918"; he was co-editor of the supplement to this work, "Veröffentlichungen aus der Gewerbe- und Konstitutionspathologie."

Aschoff was in many ways, as Garrison has called him, "the leading spirit of recent pathology," and yet the position that he will eventually occupy in twentieth century medicine is unusually difficult to estimate. Well trained in German methods of study and administration, his energetic spirit made the Pathological Institute at Freiburg a leading center for many years. Thither went pupils from many countries, some of whom worked out his ideas and later took home German methods. More, however, found so many competitors for the small amount of material available and were so seldom able even to speak with the "Professor" that they would have been better off elsewhere, especially as the assistants responsible for many details were apt to be of inferior caliber. When one recalls that many German leaders purposely picked docile assistants to carry out the master's tasks, it is perhaps significant that few of Aschoff's juniors accomplished anything noteworthy after leaving Freiburg.

A man of great intellectual vigor, his many activities required a full and methodically carried out daily program. He began work early and took less time off at midday than was customary among German intel-

lectuals, accomplishing many hours of work per week; the amount of time allotted to diversions was based mainly on standards of health and efficiency. On many occasions, as on one in 1937, his prelecture remarks in an adjoining room on the gross specimens of the day were condensed into the time required to walk rapidly past the tables on which the specimens stood. As one might expect, Aschoff's lectures were dogmatic, concerned with little but pathologic anatomy, and that of the German variety, but were clear, authoritative and not inadequate in their field.

Personally, Aschoff was a man of his times. He was one of the ninety-three German professors "well known for scientific or artistic achievements" who signed the notorious manifesto of October 1914, denying that Germany, *inter alia*, had "violated the neutrality of Belgium, assailed a single Belgian citizen without the compulsion of most bitter necessity, disregarded the principles of international law, raged brutally against Louvain," and so on—pledging their names and honor that the witness borne against Germany was false. Obviously, the *Kultur* that volunteered such a statement must have been singularly deficient in factual information or in scientific regard for the truth. It is said that for some years after the war he required of those wishing to work in his laboratory a signed statement acknowledging Allied culpability for the war. Later, however, he was courteous to foreign visitors, and especially so to Americans after his visit to this country. It has been told me by visitors to Freiburg that Aschoff was opposed to the Nazi ideology and methods but at that time was so near the retiring age that he did not wish to do anything that would disturb his retired leisure in Freiburg, where he had lived so long. I have not been able to find any published statements by him that would throw light on his attitude toward this problem.

Thus passes a leading figure of the twilight period of modern German medical science.

EDWARD B. KRUMBHAAR, M.D.

## Book Reviews

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**A Short History of Cardiology.** James B. Herrick, M.D., emeritus professor of medicine, Rush Medical College, and consulting physician to the Presbyterian Hospital, Chicago. Pages 258, with 48 figures. Price \$3.50. Springfield, Ill., and Baltimore, Md.: Charles C Thomas, Publisher, 1942.

This small volume of approximately 250 pages is well worth the reading, whether one is a medical student or a physician engaged either in general practice or in internal medicine. It is an excellent survey not only of the development of present knowledge of the heart and its diseases but of much of internal medicine as well, not only for the beginner but also for the doctor already interested in and somewhat acquainted with the history of medicine. The reviewer himself, for example, has acquired many interesting bits of information about physicians, especially those of the nineteenth century, who made contributions to cardiovascular knowledge of which he had had little or no clear idea before; in particular he would like to list the following names: Hippolito Albertini (1672-1733), who was apparently the first to emphasize the value of palpation over the heart; Allan Burns (1781-1813), who emphasized the coronary origin of angina pectoris and ascribed it to myocardial ischemia; Antonio Testa (1756-1814), who wrote a three volume work on the heart in which he devoted several pages to angina pectoris; Friedrich Kreysig (1770-1839), who also wrote a large work on the heart in which he accepted the coronary artery theory of angina pectoris, a theory which, despite these individuals, stemming from Jenner in the previous century, remained largely in the discard for one hundred years; Josef Skoda (1805-1881), who at the age of 35 wrote a classic article on percussion and auscultation, correcting much of Laennec's faulty interpretation of heart sounds and murmurs; Ludwig Traube (1818-1876), who was particularly interested in the relationship between renal and cardiac disease and who was able to reason back from contracted kidney to hypertrophy and dilatation of the left ventricle, relative insufficiency of the tricuspid valve and finally increased pulsation of the veins of the neck; Adams (1791-1875), who not only described the Adams-Stokes syndrome but wrote well on the physical findings in mitral stenosis and in aortic stenosis; Latham (1789-1875), who in his clinical lectures gave an excellent picture of rheumatic endocarditis and pericarditis with a statistical study of 136 cases of acute rheumatism, and also treated of enlargement of the heart, recognizing the variety of causes; Bouillaud (1796-1881), who established on a firm basis the relationship of acute inflammatory rheumatism and heart disease; Virchow (1821-1902), who not only initiated interest in cellular pathology but introduced in almost a modern way knowledge of thrombosis and embolism; Kirkes (1823-1864), who emphasized the occurrence of peripheral embolism from intracardiac thrombosis, especially vegetations from the cardiac valves; Francis Welch, who in 1875 clearly differentiated for the first time between the aortic lesions of atheroma and those due to syphilis; Quain (1816-1889), who in a comprehensive article entitled "Fatty Degeneration of the Heart" recognized myocardial lesions resulting from coronary obstruction; Marshall Hall (1790-1857), who in 1842 expressed the belief that sudden death was often due to arrest of the coronary circulation, and Huber, who in 1882 clearly described myocardial fibrosis as of coronary origin with a course that is not only chronic but is interrupted by explosions of so-called "heart strokes" and establishment in some cases of intracardiac mural thrombi and in others the occurrence of death from rupture of the heart wall.

One finds in the book ample mention of the better known contributions by the better known pioneers, including Galen, Leonardo da Vinci, Vesalius and Harvey, Lower and Mayow, Vieussens and Lancisi, Hales, Senac, Morgagni, Heberden and Withering, Jenner and Parry, Corvisart and Laennec, Williams and Hodgson,

Cheyne, Corrigan, and Stokes, Rokitansky, DaCosta, Potain and Vaquez, Osler, Wenckebach, Mackenzie and Lewis. There are naturally a few gaps, but astonishingly few. The reviewer would like to have seen more reference to that gold mine of cardiovascular pathologic data, brief though they were, buried in the depths of the *Sepulchretum* of Theophile Bonet, both the first edition of 1679 and the second of 1700. Morgagni's contribution really consists of a much improved and elaborated third edition of the *Sepulchretum*. Bonet had broken the ground and had revealed the wealth of the hidden treasure, which Morgagni presented in full luster to the gaze of his own generation and of those that followed.

The next to the last chapter, devoted to coronary disease, is of particular interest in its attempt to explain the lapse in interest in the subject between 1800 and 1912. The reasoning, it seems to me, is not wholly adequate, being based as it is on the distraction of the medical mind by so many other interesting new ideas and methods of study; there should not have been such complete distraction from so important a subject. Sudden deaths and temporary invalidism from heart disease were occurring commonly throughout that century, pathologists were finding scars in the heart and other evidences of serious coronary disease, but there was no clinician to put all the facts together until Herrick himself analyzed the problem. The occasional references in the literature to which he himself refers, although interesting, and of course accumulative, nevertheless do not take away in my mind at all from the luster of Herrick's own accomplishment in his classic paper published in 1912. The only outstanding omission in the book as I see it is this lack of recognition of the importance of his own contribution. It undoubtedly is from sincere modesty. Nevertheless, I am sure that a page telling of Herrick's accomplishment should be added to that chapter by the mind's eye of the reader who knows something of the work of the last generation on this subject. Some one other than Herrick himself will have actually to add this page.

The book is clear and accurate, with good style, and is obviously based on much study. One might wish that more had been written in the earlier part of the book concerning the very important century from 1650 to 1750, during which heart disease was actually discovered. One might wish that there were more about the social and the political aspects of the times for correlation with the medical contributions, but all this would have made the book not a short history of cardiology but a long one. One could wish that Dr. Herrick would sit down some time and amplify this short history, at least into a medium-sized one. He would do a great service as he has already done in presenting the shorter one.

It is difficult to know what is the best method of presentation of medical history, as he himself notes at the beginning, whether by period or by person or by subject. He uses all three in the course of the book, ending it with several chapters on specific subjects. All these methods of attack are interesting and worth while, and might be worked out more completely even in the same volume; for example, the last part of chapter 4 in this book, which is entitled "Laennec to Virchow," concerns rheumatic heart disease, which, with such a label, could be separated off as a separate chapter like those that follow at the end of the book. There might be chapters on cardiac enlargement, on arrhythmia and on heart failure. However, such amplification, as already said, would lead to a large volume, and this Dr. Herrick did not intend to undertake.

In summary, then, the reviewer would express his gratitude to Dr. Herrick for what he has done and express the hope that he will do more.

**Central Autonomic Regulations in Health and Disease (with Special Reference to the Hypothalamus).** Heymen R. Miller, M.D., associate attending physician, Montefiore Hospital, New York. Introduction by John F. Fulton, M.D., Sterling professor of physiology, Yale University. Cloth. Pages 440, with bibliography, index and 64 illustrations. Price \$5.50. New York: Grune and Stratton, 1942.

In this book there is a passage which reads: "The total effect of the normal constant 'tonic' autonomic discharge is to confer upon the body a coordinated dynamic equilibrium maintained at an optimal standard. Without this coordination



the multitudinous and complex reactions of the autonomic nervous system would become incongruous and a kaleidoscopic helter-skelter" (pages 19 to 20). It is not too fanciful to say that the author has performed for one's understanding of the autonomic nervous system a feat of coordination comparable to that which this system performs for the body. He has brought a semblance of order out of what to most physicians not neurologically trained has been chaos. That order does not reign supreme even now is due to the still great gaps in knowledge of this field and to the many points on which the evidence is in conflict.

The book emphasizes function rather than structure. For example, roughly five sixths of its contents are devoted to normal and pathologic physiology, with the anatomic sixth unconventionally but conveniently placed at the end. The first chapter, serving to orient the reader, deals with the general physiology of the autonomic system. Subsequent chapters are concerned with the regulation of body temperature, water and minerals, metabolism, the sleep-waking rhythm, the mechanism of emotions and the major organ systems. The material is logically organized, thoroughly documented and well illustrated by photographic reproductions and clear, relatively simple charts and drawings. Evidence is impartially presented, and conclusions are carefully drawn. There is no dogma. The hypothalamus receives a great deal of attention. This, as the author states, has been deliberate, and most readers will add, fortunate in view of the growing recognition of the importance of this structure as an originator, mediator and receptor of autonomic impulses.

The introductory chapter, the discussion of diabetes insipidus and the section on the sleep-waking rhythm are particularly good. The chapter on metabolism is less so. While many clinical conditions influenced by the autonomic nervous system are discussed, it is, in the reviewer's opinion, regrettable that little or no space is given to such common disturbances as postural hypotension, vasomotor instability and migraine, in the genesis of which this system must surely play a large role. The occasional brief case reports, interposed to demonstrate the application to disease processes of various neurophysiologic principles, are usually apt and instructive. Some, however, are unconvincing either by reason of insufficient definitive data or because the relation of the autonomic system to the clinical phenomena described is assumed rather than proved.

Despite its shortcomings, the book represents a difficult job unusually well done. Its readers will be rewarded with pleasure and profit.

**Surgical Pathology.** William Boyd, M.D., LL.D., M.R.C.P.Ed., F.R.C.P. Lond., Dipl. Psych., F.R.S.C., professor of pathology, University of Toronto. Fifth edition, thoroughly revised. Pages 843, with 502 illustrations and 16 colored plates. Price \$10. Philadelphia and London: W. B. Saunders Company, 1942.

The usefulness of this work is well established and warrants fully the revision the book has received. A good deal of new subject matter has been introduced, including a new chapter on the surgical pathology of the chest, in which are discussed lesions of the trachea and bronchi, the lungs, the mediastinum, the pleura and the pericardium. The references at chapter ends have been rearranged according to subjects. Many microscopic drawings have been replaced by photographs. With only few exceptions, the illustrations, both gross and microscopic, are attractive and instructive. The first nine chapters deal with subjects of general interest, such as inflammation, shock, hemorrhage and tumors. The chapter on tumors is comprehensive and adequate. The "radiosensitivity" of different cancers is discussed with commendable cautiousness. On page 109 Hansemann is misspelled Hausemann. The word "cancer" is used as synonymous with "carcinoma" and not as including both sarcoma and carcinoma, which is now the common usage. The larger part of the book by far is devoted to the description of processes and conditions in the organs, of special surgical interest. Certain omissions may be mentioned, e. g., anomalies and defects (diaphragmatic hernia), carcinoma of the larynx and tumors of the maxillary sinuses. Carcinoma of the mouth and pharynx, as well as that of the bronchi, is well described, but not one

word is said about carcinoma of the larynx. So far as it goes, the book achieves its object, which is "to present those aspects of pathology which will prove useful to the surgeon."

**Changes in the Knee Joint at Various Ages with Particular Reference to the Nature and Development of Degenerative Joint Disease.** Granville A. Bennett, M.D., associate professor of pathology, Harvard Medical School; Hans Waine, M.D., research fellow in medicine, Harvard Medical School, graduate assistant in medicine, Massachusetts General Hospital; Walter Bauer, M.D., associate professor in medicine, Harvard Medical School, physician to the Massachusetts General Hospital, director, Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases. Pp. 97, with 31 plates. Price \$2.50. New York: The Commonwealth Fund, 1942.

As indicated by the title, this monograph describes the morphologic conditions of the knee joint at various ages. It is based on a thorough study of knee joints from a number of subjects in each decade of life who gave no history or clinical evidence of joint disease. The photographs illustrate well the appearances, gross and microscopic, of the joint in each of the nine decades. The main outcome of the study is that after the second decade the joint cartilages show degenerative changes which become more and more marked as age advances. These changes are similar to those in so-called hypertrophic arthritis, which the authors properly prefer to call degenerative joint disease and which they regard to be "in essence, primarily a degenerative process of hyaline cartilage," the intimate nature and causes of which invite further investigation.

**The Care of the Aged (Geriatrics).** Malford W. Thewlis, M.D., attending specialist in general medicine at the United States Public Health Hospitals, New York. Fourth edition. Cloth. Pp. 589, with 50 illustrations. Price \$7. St. Louis: C. V. Mosby Company, 1942.

**Problems of Ageing, Biological and Medical Aspects.** Second edition. Thirty-seven contributors. Edited by E. V. Cowdry, Washington University, St. Louis. Pp. 936, with 129 figures. Price \$10. Baltimore: Williams & Wilkins Company, 1942.

The publication of the fourth edition of the "Care of the Aged (Geriatrics)" by Malford W. Thewlis and of the second edition of "Problems of Ageing" indicates an actively increasing interest in the biologic and the practical aspects of aging. The fact that the book by Thewlis has reached its fourth issue shows that it meets the needs of physicians for a practical guide in the care of aged patients. "Problems of Ageing" was published first in 1939, and a descriptive review of it appeared in the *ARCHIVES OF PATHOLOGY* (28:280, 1939). A reprint was issued in 1940. The second edition, revised and enlarged, has nine new chapters and the number of pages has grown from 749 to 936. Each chapter is a unit by itself, with summary and references. These new editions mark definite progress in the study of aging as well as of the care of the aged.

## Books Received

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SHOCK, ITS DYNAMICS, OCCURRENCE AND MANAGEMENT. Virgil H. Moon, M.Sc., M.D., professor of pathology, Jefferson Medical College, Philadelphia. Pp. 324, with 36 figures. Price \$4.50. Philadelphia: Lea & Febiger, 1942.

A HANDBOOK OF ALLERGY FOR STUDENTS AND PRACTITIONERS. Wyndham B. Blanton, M.D., Litt.D., professor of clinical medicine and chief of the immunology clinic, O.P.D., Medical College of Virginia, Richmond, Va. Pp. 190, with 20 figures. Price \$3. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

HUMAN PATHOLOGY. Howard T. Karsner, M.D., professor of pathology, Western Reserve University, Cleveland. Sixth edition, completely revised and reset. Pp. 817, with 460 illustrations and 16 color plates. Price \$10. Philadelphia: J. B. Lippincott Company, 1942.

A SHORT HISTORY OF CARDIOLOGY. James B. Herrick, emeritus professor of medicine, Rush Medical College; consulting physician to the Presbyterian Hospital, Chicago. Pp. 258, with 48 illustrations. Price \$3.50. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

OUTLINE OF HISTOLOGY. Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., New York University, Departments of Anatomy, College of Dentistry and Graduate School of Arts and Science. Pp. 112, with 56 figures. Price \$2.50. St. Louis: C. V. Mosby Company, 1942.

INTRODUCTION TO PARASITOLOGY. A. S. Pearse, professor of zoology, Duke University. Pp. 357, with 448 figures. Price \$3.75. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

A CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY. Israel Davidsohn, M.D., director of laboratories and pathologist, Mount Sinai Hospital, Chicago, and assistant professor of pathology, University of Illinois College of Medicine. Second edition, revised. Pp. 47. Price \$1.75. Board of Registry, American Society of Clinical Pathologists, 1942.

PROBLEMS OF AGEING, BIOLOGICAL AND MEDICAL ASPECTS. Second edition. Thirty-seven contributors. Edited by E. V. Cowdry, Washington University, St. Louis. Pp. 936, with 129 figures. Price \$10. Baltimore: Williams & Wilkins Company, 1942.

BLOOD SUBSTITUTES AND BLOOD TRANSFUSION. Edited by Stuart Mudd, M.D., professor of bacteriology, University of Pennsylvania School of Medicine, Philadelphia, and William Thalhimer, M.D., director, Human Serum Division, Public Health Research Institute of the City of New York, Inc. Pp. 407. Price \$5. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

## MORPHOLOGIC CHANGES IN THE HUMAN KIDNEY FOLLOWING PROLONGED ADMINISTRATION OF ALKALI

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In the clinical studies of alkalosis previously reported<sup>1</sup> evidence was presented indicating that although the urea clearance frequently diminished during alkalosis, renal function invariably returned to its original level when the acid-base balance was restored. It was further shown that prolonged ingestion of alkali likewise did not permanently lower renal function. On the basis of these observations it did not appear likely that either alkalosis or prolonged alkali therapy produced irreversible anatomic renal injury. This view was supported by the absence of significant anatomic changes in the kidneys of the dog after long-continued administration of large quantities of sodium bicarbonate.<sup>2</sup> To secure additional information on this problem, we undertook a histologic study of the kidneys of patients who had received large amounts of alkali and who had died either in alkalosis or of other complications.

Few studies have appeared in the literature describing the effects of alkali therapy and of alkalosis complicating antacid treatment on the structure of the human kidney.

Hardt and Rivers<sup>3</sup> reported the case of a 66 year old man who had taken sodium bicarbonate intermittently for fifteen years for distress caused by ulcer. After he had been in the hospital under intensive alkali therapy for fifteen days, he became stuporous and subsequently died despite the use of saline infusions. The plasma carbon dioxide had measured 44.9 and 50.4 millimols per liter, and the

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1. Kirsner, J. B., and Palmer, W. L.: *J. A. M. A.* **116**:384, 1941; *Arch. Int. Med.* **69**:789, 1942.

2. Kirsner, J. B.: *Arch. Path.* **32**:76, 1941.

3. Hardt, L. L., and Rivers, A. B.: *Arch. Int. Med.* **31**:171, 1923.

blood urea nitrogen 134 and 212 mg. and the blood creatinine 4.3 mg. per hundred cubic centimeters. Necropsy revealed a duodenal ulcer, bilateral bronchopneumonia and generalized arteriosclerosis. Microscopic examination of the kidneys disclosed focal and diffuse interstitial lymphocytic infiltration, sclerosis of some glomeruli and complete hyalinization of others, thickening of most of Bowman's capsules, and sclerosis of the arteries and arterioles.

Cooke<sup>4</sup> reported the case of a 46 year old man who had taken alkalis for one year. The patient had been vomiting for five weeks and was admitted to the hospital in a semicomatose condition. After the administration of saline solution the serum carbon dioxide decreased from 56.2 to 31.8 millimols per liter and the plasma sodium chloride rose from 353 to 546 mg. per hundred cubic centimeters. The blood urea nitrogen increased, however, from 165 to 252 mg. per hundred cubic centimeters, and the patient died within several days. The kidneys were large, smooth and engorged with blood. The microscopic changes were interpreted as indicative of "chronic interstitial nephritis" with terminal acute nephritis.

Oakley<sup>5</sup> described the anatomic findings in 2 patients who died in alkalosis after alkali therapy: 1. A 63 year old man with a duodenal ulcer had been treated with a mixture of magnesium carbonate, sodium bicarbonate and bismuth oxy-carbonate. The patient had received 90 Gm. of sodium bicarbonate in four days, at the end of which time he became irrational and comatose; he died four days later. The blood urea nitrogen had measured 140 mg. per hundred cubic centimeters. The kidneys were normal grossly, and the only microscopic changes recorded were swelling and focal fatty degeneration of the tubular epithelium. 2. A 64 year old man, treated similarly, had received 541 Gm. of sodium bicarbonate in eleven days. Symptoms of alkalosis appeared on the eleventh day, and the patient died three days later. The blood urea nitrogen twenty-four hours before death was 250 mg. per hundred cubic centimeters; the urine was reported as normal. Microscopic study of the kidneys revealed only a slight amount of fibrosis, occasional arteriosclerotic changes and a few small calcareous deposits.

#### METHOD OF STUDY

The present investigation comprises a study of the autopsy observations in 14 patients who had received during life large quantities of alkali and for whom adequate studies of acid-base balance were available. The series is composed of (group 1)) 4 patients who had taken large amounts of alkali but in whom alkalosis had never occurred; (group 2) 6 patients who had experienced one or more episodes of alkalosis during alkali therapy but who did not die in alkalosis (5 of this group died of other complications several weeks after alkalosis, while the sixth patient died ten months after severe alkalosis), and (group 3) 4 patients who had taken large quantities of alkali and in whom alkalosis was present at the time of death.

#### RESULTS

##### *Group 1. Prolonged Alkali Therapy—No Alkalosis.*

CASE 1.—A 52 year old man had been treated for symptoms of ulcer for seven years, during which time he had received large amounts of alkali. He entered the hospital because of a severe hemorrhage, which proved fatal on the twenty-

4. Cooke, A. M.: *Quart. J. Med.* **1**:527, 1932.

5. Oakley, W. M.: *Lancet* **2**:187, 1935.

ninth day despite six blood transfusions and the subcutaneous administration of a 5 per cent solution of dextrose and a physiologic solution of sodium chloride. Five hundred and eighty-five grams of sodium bicarbonate and calcium carbonate was administered during the first fifteen days; the patient received no alkali during the last fourteen days. The acid-base balance was normal. There was no elevation of blood urea or of nonprotein nitrogen, and the urea clearance was 69 per cent of average normal. The urine contained hyaline and granular casts.

Necropsy revealed the following conditions: a benign peptic ulcer of the lesser curvature of the stomach with recent thrombosis of the artery in the floor of the ulcer; acute distention of the right side of the heart with acute generalized passive hyperemia, most evident in the kidneys; pulmonary edema; right hydrothorax; ascites; moderate sclerosis of the aorta and coronary arteries.

The kidneys were purplish and firm; the right weighed 185 Gm. and the left 160 Gm. The capsule of each stripped easily, leaving a smooth surface. The parenchyma was cyanotic. Microscopic examination revealed only mild arteriosclerotic changes.

CASE 2.—A 56 year old man had suffered from high blood pressure for at least four years. One year, five months, and one month previous to his admission to the hospital he had experienced hemorrhages arising from a gastric ulcer. Alkali had been taken intermittently during this time. He entered the hospital with another massive hemorrhage. The therapy included two blood transfusions and 1,521 Gm. of sodium bicarbonate and calcium carbonate given over a period of thirty-nine days. The acid-base balance was normal. The blood urea nitrogen was 9.6 mg. per hundred cubic centimeters and the urea clearance 89 per cent of average normal; nine examinations of the urine gave negative results. The patient returned two months later because of a recurrence of the gastric ulcer, hypertensive vascular disease and hypertensive retinitis. Alkali had been taken occasionally. The blood pressure then was 188 systolic and 120 diastolic. Three hundred and ninety-three grams of sodium bicarbonate and calcium carbonate was administered in nine days. The acid-base balance remained normal; the blood urea nitrogen measured 14 mg. per hundred cubic centimeters. Six specimens of urine were normal. One year after the initial entry the patient returned for a third time. He received, over a period of fifty-nine days, 2,301 Gm. of sodium bicarbonate and calcium carbonate. The blood urea nitrogen amounted to 12.3 mg. per hundred cubic centimeters, and the urea clearance was 94 per cent of average normal. The lesion of the stomach discovered at this time was diagnosed as carcinoma. The patient died several months later.

Necropsy revealed diffuse carcinomatous infiltration of the stomach with pyloric obstruction. There was a healed benign gastric ulcer in the prepyloric region, as well as a chronic benign ulcer high on the lesser curvature, with fixation to the tail of the pancreas. Other conditions were: bronchopneumonia of the right upper pulmonary lobe and an early abscess in the right lower lobe, bilateral hydrothorax and arteriosclerosis of the aorta.

Each kidney weighed 140 Gm. and was flabby in consistency. The capsule stripped readily, leaving a uniformly granular pale purple-gray surface. A few small cysts were present on the surface. The cortex measured 3 to 4 mm.; the corticomedullary demarcation and the cortical markings were obscured. Several small cysts were located in the medulla. The renal pelves and ureters were normal. Microscopic examination revealed a few areas of scarring with foci of round cells. Some of the glomeruli showed varying stages of hyalinization; the associated tubules were atrophied. The tubules, especially those in the cortex, showed

moderate fatty changes and contained protein debris and hyaline casts. There was some increase in interstitial fibrous tissue. The arteries and arterioles showed varying degrees of sclerosis.

CASE 3.—A 62 year old man had experienced gastrointestinal distress for ten years. Alkali had been taken regularly for about one year and then intermittently during the subsequent nine years. He entered the hospital because of a severe hemorrhage, and for the initial five days was treated with alkali, receiving 320 Gm. of calcium carbonate. Six blood transfusions were given. Subtotal gastrectomy was performed because of persistent massive hemorrhage. The patient continued to bleed, however, and he died shortly after the operation. There was no alkalosis.

Necropsy revealed the following conditions: a large chronic benign ulcer on the posterior wall of the stomach near the cardiac portion and close to the greater curvature; perforation of an aneurysm of a short gastric artery located in the floor of the ulcer; marked parenchymatous changes in the myocardium with moderate dilatation; focal necrosis of the liver; marked bilateral hypostatic pulmonary edema with aspirated blood and probably beginning bronchopneumonia.

Each kidney weighed 130 Gm.; the surrounding fat and capsule stripped easily. The capsular surfaces were smooth except for a few tiny retention cysts. The vascular markings were fairly well preserved. The renal pelves were normal. The chief microscopic observations were scattered hyalinized glomeruli and slight fibrous thickening of the walls of the larger arteries. Occasional cortical tubules showed fatty degeneration.

CASE 4.—A 57 year old man had experienced gastrointestinal distress for fifteen years. One year prior to entry he had been treated with moderate amounts of alkali following the appearance of tarry stools. Sodium bicarbonate had been taken occasionally for three weeks prior to entry. The patient received a total of 1,212 Gm. of sodium bicarbonate and calcium carbonate over a period of twenty-three days. He died on the twenty-fourth day as a result of coronary thrombosis. The acid-base balance was normal. The blood urea nitrogen was 14.8 mg. per hundred cubic centimeters and the urea clearance 78 per cent of the average normal. One of ten specimens of urine contained albumin.

Necropsy revealed the following conditions: multiple healed infarcts of the myocardium; marked coronary sclerosis with recent rupture of a plaque; peptic ulcer of the first part of the duodenum; cardiac hypertrophy and dilatation; congestion of the liver and spleen; nodose goiter with solitary cyst.

The kidneys each weighed 240 Gm. and were very firm. The capsules stripped easily, leaving smooth, pale red surfaces with a few cysts, measuring up to 5 mm., and small irregular stellate depressed scars. The cut surfaces appeared cyanotic. The cortex of each kidney was 7 mm. wide; the corticomedullary demarcation was distinct, although the other markings were not clear. Microscopic examination revealed scattered hyalinized glomeruli and focal round cell infiltration. Protein debris was present in the lumens of the tubules. The epithelium of the tubules contained small amounts of fat. There were moderate sclerosis of the arteries and marked dilatation and congestion of the capillaries.

No significant renal changes were noted in these 4 cases despite the prolonged use of considerable quantities of alkali. The hyalinized glomeruli and atrophied tubules observed in cases 2 and 4 are usual autopsy findings at this age. The focal fatty degeneration of the tubular

epithelium and the protein precipitate and hyaline casts observed in 2 cases are acute changes attributable to the passive congestion and anemia.

*Group 2. Prolonged Alkali Therapy with Alkalosis—Death Caused by Other Complications.*

CASE 1.—A 60 year old man had experienced distress from a peptic ulcer for four years. He occasionally took sodium bicarbonate and frequently induced vomiting to obtain relief from pain. He entered the hospital because of a severe hemorrhage and died sixteen days later despite eight blood transfusions. Six hundred and ninety-five grams of sodium bicarbonate, calcium carbonate and tricalsate<sup>6</sup> was administered during this period. Studies of the acid-base balance revealed moderate alkalosis on the third day (carbon dioxide 36.6 millimols per liter and  $p_H$  7.53); this became severe on the seventh day with the serum carbon dioxide measuring 46.3 millimols per liter and the  $p_H$  7.63. The alkalosis subsided rapidly after the discontinuance of the alkali therapy and with the administration of ammonium and sodium chloride by mouth. Alkali therapy (tricalsate) was resumed on the tenth day, but the acid-base balance remained normal. The blood urea nitrogen, which had risen to 46.8 mg. per hundred cubic centimeters on the eighth day, decreased to 19.4 mg. one week later. The urea clearance values ranged from 40 to 79 per cent of average normal. The blood creatinine fluctuated between 1.29 and 1.88 mg. Seven examinations of the urine gave negative results.

Necropsy revealed a massive hemorrhage into the stomach and duodenum from a branch of the superior pancreaticoduodenal artery located in the floor of a large duodenal ulcer; the lungs and retroperitoneal tissues were markedly edematous and both pleural cavities contained fluid.

The kidneys were embedded in considerable adipose tissue; together they weighed 320 Gm. The capsule of each stripped easily, leaving a smooth surface. The cortices were extremely pale and measured 5 to 6 mm.; the medullae measured 15 mm. The cortical markings were fairly distinct. The renal pelves were normal. Microscopic examination revealed most of the glomeruli to be normal; fat stains demonstrated a few foci of slight fatty degeneration in the tubules. There was moderate intimal thickening of the larger arteries with extreme thickening of some arterioles.

CASE 2.—A 54 year old man had experienced distress from ulcer for eighteen years, for which sodium bicarbonate had been taken frequently. He was hospitalized for twenty-one days and during the first nine days received 137 Gm. of sodium bicarbonate and calcium carbonate. Symptoms of alkalosis appeared on the ninth day. The serum carbon dioxide at this time was 46.5 millimols per liter and the  $p_H$  7.58. The blood urea nitrogen increased from 9.2 mg. to 18.1 mg. per hundred cubic centimeters and the urea clearance, previously 63 per cent of average normal, decreased to 37 per cent. Alkalis were stopped for six days, and sodium and ammonium chloride were given by mouth. The acid-base balance returned to normal in six days; the blood urea nitrogen decreased to 10.9 mg. per hundred cubic centimeters, and the urea clearance increased to 65 per cent. Tricalsate then was substituted for the previous alkali, and the patient's subse-

6. Tricalsate is a preparation which when in aqueous suspension consists essentially of tribasic calcium phosphate and sodium citrate.



quent clinical course was uneventful. He died ten months later, however, after an operation for carcinoma of the gallbladder.

Necropsy revealed carcinoma of the gallbladder with metastases to the liver and perihepatic lymph nodes, hemorrhage into the duodenum from a duodenal ulcer and bilateral pulmonary edema.

The kidneys were of about equal size and together weighed 300 Gm. The capsules stripped easily leaving smooth surfaces. The cortices were slightly brown; the cortical markings were preserved. Microscopically the glomeruli were normal. There was no significant change in the tubular epithelium. The only microscopic change was slight diffuse subcapsular scarring with round cell infiltration.

CASE 3.—A 38 year old man had had a stenosing duodenal ulcer for fifteen years complicated by a massive hemorrhage eight years previously. He had vomited frequently since that time and often had required sodium bicarbonate for the relief of his distress. The blood pressure was found to be 190 systolic and 130 diastolic. Alkali, mainly sodium bicarbonate, was given to the amount of 1,292 Gm. during the first seventeen days. Partial gastrectomy was performed on the eighteenth day because of obstruction. The patient vomited persistently and died several days later despite the parenteral administration of large quantities of physiologic solution of sodium chloride and 5 per cent dextrose solution. The serum carbon dioxide and  $p_{\text{H}}$  were elevated slightly on the fiftieth hospital day; the blood urea nitrogen was 25.5 mg. per hundred cubic centimeters and the urea clearance 39 per cent of average normal. Two of six specimens of urine contained hyaline and granular casts, albumin and occasional red and white blood cells.

Necropsy revealed the following conditions: a chronic duodenal ulcer with adherence to the pancreas; dilatation of the duodenum; torsion and gangrene of the proximal anastomosed jejunal loop with dilatation, hemorrhage and venous thrombosis; hemoperitoneum; cardiac hypertrophy.

The kidneys each weighed 160 Gm.; they were markedly pale, gray-white and firm. The capsules stripped easily, leaving smooth surfaces. The cortices measured 5 to 6 mm.; the cortical markings were distinct. On microscopic examination, many of the glomeruli showed varying degrees of hyalinization, and some were partially or completely adherent to the capsules. There was marked thickening of Bowman's capsules. The epithelium of some of the collecting tubules showed mild degenerative and regenerative changes,<sup>7</sup> and the lumens of these tubules contained blue-staining masses (with the hematoxylin and eosin stain) suggesting calcium. Protein precipitate was noted occasionally. There was a definite increase in the interstitial fibrous tissue; scattered foci of round cells were present. Both the large and the small renal arteries showed intimal proliferation.

CASE 4.—A 34 year old man had experienced symptoms of ulcer for five years, during which time he had taken moderate amounts of sodium bicarbonate. Hypertension had been present for at least six years. He entered the hospital with a severe hemorrhage and received, over a period of forty-nine days, 2,700 Gm. of alkali, consisting of sodium bicarbonate, calcium carbonate and tricalate. Studies

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7. Regenerative changes accompanying degeneration of the epithelium of the renal tubules are frequently observed. Experimentally, they have been noted to occur within a period of seven days.

of the acid-base balance revealed mild alkalosis. Renal function was impaired, the urea clearance values ranging from 26 to 47 per cent of average normal. The blood urea nitrogen rose to 43.8 mg. per hundred cubic centimeters but gradually decreased by the forty-fourth day to 12.3 mg. After recovery from the hemorrhage the patient was continued on a modified ulcer regimen with tricalsalate for five months. At this time he experienced a second severe hemorrhage, from which he died on the thirty-second day. Twenty-four hours before death the patient experienced a transfusion reaction characterized by a chill and a rise of temperature to 106 F. Treatment had included the administration of small amounts of sodium bicarbonate, subcutaneous injection of physiologic solution of sodium chloride and 5 per cent dextrose solution, and nine blood transfusions. There was no evidence of alkalosis at this time. The blood urea nitrogen, however, ranged from 40.2 to 113.2 mg. per hundred cubic centimeters and the urea clearance from 9 to 24 per cent of average normal. The blood creatinine varied from 2 to 3 mg. per hundred cubic centimeters. Five of nine specimens of urine contained a trace of albumin; the maximum specific gravity was 1.018.

Necropsy revealed the following conditions: an acute duodenal ulcer with evidence of a recent massive hemorrhage; a chronic healed duodenal ulcer; marked cardiac hypertrophy, especially of the left ventricle; marked pulmonary edema with early bronchopneumonia; parenchymatous degeneration of the liver and of the myocardium; moderate sclerosis of the aorta and of the coronary and medium-sized arteries.

The right kidney weighed 90 Gm. and the left 110 Gm.; they were flabby and pale. The capsule of each stripped easily, leaving a finely granular surface with numerous punctate hemorrhages. The cortices were somewhat decreased in width, and the cortical markings were indistinct. The renal pelves were normal. Microscopic examination revealed a marked increase in fibrous tissue with diffuse and focal round cell infiltration. Many of the glomeruli showed varying degrees of hyalinization. Many of Bowman's capsules were thickened. These changes were associated, especially in the cortices, with marked dilatation of tubules in groups, the epithelium of which was flat and cuboidal. Hyaline casts were noted frequently. No calcium precipitate was visible. Fat stains disclosed focal fatty degeneration of the tubular epithelium. The arteries and arterioles were markedly sclerosed, and the walls of the arterioles showed severe fatty degeneration.

CASE 5.—A 57 year old man entered the hospital because of massive hemorrhage from a gastric ulcer. No alkali had been taken previously. He remained in the hospital for sixty-six days, during which time he received a total of 2,700 Gm. of sodium bicarbonate and calcium carbonate. Nine examinations of the urine gave negative results. The serum carbon dioxide amounted to 33.6 millimols per liter and the  $p_H$  was 7.58 on the seventh hospital day. He returned three and one-half years later with a recurrence of the ulcer; alkalis had been taken during the interim. The patient received 2,574 Gm. of sodium bicarbonate and calcium carbonate over a period of sixty-six days. Studies of the acid-base balance on the third, eleventh and twenty-eighth days revealed mild to moderately severe alkalosis, the serum carbon dioxide ranging from 32.4 to 37.8 millimols per liter, the  $p_H$  from 7.51 to 7.63 and the chloride from 90.6 to 98.2 millimols per liter. The blood urea nitrogen varied from 15.8 to 20.8 mg. per hundred cubic centimeters and the urea clearance from 41 to 109 per cent of the average normal. In eleven examinations the urine was normal. Six months later (four years after

the initial entry) the patient was hospitalized because of a recurrence of hemorrhage; alkalis had been taken occasionally during this interval. He remained in the hospital for two hundred and eleven days, receiving a total of 6,933 Gm. of alkali (mainly calcium carbonate), which was almost entirely administered during the first one hundred and seventy-three days. Aspiration of the gastric contents yielded from 300 to 400 cc. nightly. Many determinations of the acid-base balance and of renal function (urea clearance) were made, as shown in table 1.

Twenty-seven examinations of the urine gave negative results; the maximum specific gravity was 1.030. During the last eighty days in the hospital the patient's clinical course was complicated by the development of auricular flutter. He also experienced severe bouts of coughing. Cyanosis, which had been present to a mild degree, increased. The treatment included parenteral administration of a 5 per cent dextrose solution and physiologic solution of sodium chloride and blood

TABLE 1.—*Acid-Base Balance and Urea Clearance in Case 5*

Hospital Day	Serum Cl, mM per Liter	Serum CO <sub>2</sub> , mM per Liter	pH	Blood Urea N, Mg. per 100 Cc.	Urea Clearance, % Av. Normal
120th.....	100.2	39.0	7.53	15.1	79
137th.....	92.1	36.1	7.52	14.9	91
141st.....	90.5	35.3	7.46		
152d.....	84.3	45.8	7.53		
153d.....	....	43.2	7.48	19.7	
162d.....	88.6	32.6	7.47	16.1	61
164th.....	100.8				
168th.....	88.4	33.4	7.47	40.4	
172d.....	93.8	30.4	7.43		
179th.....	94.1	33.5	6.50	9.2	87
183d.....	91.4	33.3	7.50	10.1	63
186th.....	....	34.0	7.50	9.1	80
190th.....	....	36.2	7.57	14.6	
193d.....	88.0	37.4	7.53	17.4	102
200th.....	89.0	35.0	7.48	25.0	73
207th.....	95.0	26.4	7.41	43.9	41
210th.....	92.3	23.4	7.33	63.5	
211th.....	Died				

transfusions. The patient, however, did not improve, and he was found dead in bed on the two hundred and eleventh hospital day.

Necropsy revealed the following conditions: marked atrophic pulmonary emphysema; senile pulmonary bullous emphysema with marked hypertrophy of the myocardium on the right (cor pulmonale); acute pulmonary edema; acute distention of the right ventricle with acute generalized passive congestion; "nutmeg" liver; chronic induration of the spleen; bilateral hydrothorax; massive benign peptic ulcer of the lesser curvature in the prepyloric area of the stomach; chronic atrophic gastritis; old perforative peptic ulcer with adhesions to the liver; moderate sclerosis of the pulmonary artery and of the aorta.

The right kidney weighed 180 Gm.; the left, 155 Gm. The cortical markings were somewhat obscured. The capsules stripped readily from smooth pale surfaces. There appeared to be an increased amount of yellowish white material in the cortices and pyramids. The renal pelves and ureters were normal. On microscopic examination, most of the glomeruli were normal. A few were hyalinized, and the corresponding tubules were atrophied. Considerable protein precipitate

was present in Bowman's spaces. The epithelium of the convoluted tubules was markedly swollen, so that the lumens were almost obliterated in occasional areas. Some of the nuclei were hyperchromatic, while others had disappeared. Occasional hyaline casts were visible in the collecting tubules. A few of the collecting tubules contained blue-staining material suggesting calcium; small foci of round cells occasionally surrounded these tubules. There was marked fatty degeneration, particularly of the collecting tubules, although some of the convoluted tubules also were affected.

CASE 6.—A 52 year old man had experienced gastrointestinal distress for three years, during which time he had had four hemorrhages. Large amounts of alkali were taken by him. The treatment included administration of 400 Gm. of calcium carbonate and magnesium oxide, given during the first twenty-two days, and aspiration of the stomach daily for the same length of time, the aspirates averaging 300 cc. in amount. On the twenty-third hospital day cholecystectomy and

TABLE 2.—*Acid-Base Balance and Urea Clearance in Case 6*

Hospital Day	Serum Cl, mM per Liter	Serum CO <sub>2</sub> , mM per Liter	pH	Blood Urea N, Mg. per 100 Cc.	Urea Clearance, % Av. Normal	Non-protein Nitrogen, Mg. per 100 Cc.	Creatinine, Mg. per 100 Cc.	Total Base, mEq. per Liter
3d	94.0	30.7	....	....	..	53.6	....	....
4th	....	....	....	40.7	15	....	....	....
10th	72.0	45.0	7.62	65.1	..	....	3.37	....
14th	86.0	38.1	7.63	45.2	10	....	....	....
18th	97.8	32.9	7.52	43.5	22	....	....	....
21st	....	30.0	7.53	38.8	16	....	....	....
23d	Posterior gastroenterostomy and cholecystectomy							
48th	98.8	24.9	7.47	17.8	22	....	....	....
60th	98.0	26.4	7.34	20.4	37	....	....	....
66th	97.6	22.8	7.32	27.3	33	....	....	138.6
73d	98.2	20.4	7.38	26.6	30	....	....	....
75th	....	16.7	....	....	..	....	....	....
77th	96.3	16.8	....	....	..	....	....	140.6
79th	....	10.1	....	....	..	42.5	....	Died

posterior gastroenterostomy were performed for cholelithiasis and an obstructing duodenal ulcer. No alkali was given postoperatively. The patient's course was complicated by infection of the wound, which was treated with sulfanilamide. The application of sulfanilamide was discontinued after four days, however, because of the toxic reaction. Large quantities of physiologic solution of sodium chloride, 5 per cent saline solution, Ringer's solution and 5 per cent dextrose solution were given parenterally. Wangensteen suction of the stomach was carried out for fourteen days postoperatively, the quantities removed varying from 500 to 3,000 cc. in amount daily. A number of specimens of urine contained albumin. Determinations of acid-base balance and renal function (urea clearance) were made frequently, as shown in table 2.

The patient's condition gradually became worse, and he died on the fifty-sixth postoperative day of bronchopneumonia.

Necropsy revealed generalized latent osteitis fibrosa cystica, hyperplasia of the parathyroid glands, pyloric stenosis, bronchopneumonia of the right lower pulmonary lobe and generalized arteriosclerosis.

The kidneys were small and pale; together they weighed 165 Gm. The capsules stripped easily from smooth surfaces. The cortical markings were obscured. The pelvic fat was increased, and each renal pelvis was dilated and thick walled.

The calices were large and clubbed on the ends. The ureters were normal. Microscopic study revealed distinctly abnormal kidneys. The tips of the pyramids were not recognizable. In the cortices many wedge-shaped areas of scarring and chronic inflammation alternated with more normal areas. The ratio of scarred to normal tissue was estimated to be 1:2. Remnants of hyalinized glomeruli and some partially hyalinized and shrunken tubules were visible in the scarred areas. The inflammatory cells of the scars consisted mostly of small mononuclears and lymphocytes. Most of the glomeruli in the more normal areas were well preserved. The tubular epithelium in the well preserved regions varied from flat cuboidal to tall cells; the nuclei varied greatly in size. Many of the tall cells contained pale, granular or vacuolated cytoplasm, and some contained colloid droplets. Casts were infrequent, and there was little protein debris. Numerous cast-filled and cast-obstructed collecting tubules were seen. Most of the casts were composed of deep blue or blue-purple staining granular masses forming dense aggregates, suggesting calcium. Similar masses were present in the interstitial tissue with no recognizable tubular membrane surrounding them. The presence of these was associated with an increase in fibrous tissue but with little inflammatory reaction. The larger arteries were moderately hyalinized but not greatly narrowed. The small arteries, especially those in the scarred regions, were thickened.

No significant renal changes were noted in cases 1 and 2 despite the previous existence of severe alkalosis. The scattered hyalinization of glomeruli and the focal fatty degeneration in the tubular epithelium are usual necropsy findings in patients of this age.

The alkalosis in cases 3 and 4 had been mild. The hypertension which had been present in both patients undoubtedly accounted for all the changes described. An additional observation in case 3 was the presence in a few collecting tubules of blue-staining material suggestive of calcium; this finding is discussed later.

Case 5 constitutes one of the most interesting of the entire series. The patient had consumed enormous quantities of alkali, receiving during three hospital periods alone 12,207 Gm. of sodium bicarbonate and calcium carbonate. Large amounts of the gastric contents had been aspirated daily, and the patient had experienced one episode each of mild, moderate and severe alkalosis. There was little anatomic evidence of chronic renal injury despite these factors. Most of the glomeruli were normal. The swelling of the tubular epithelium with focal necrosis and fatty degeneration and the presence of protein precipitate and hyaline casts are attributable to the marked passive congestion. Blue-staining material was visible in occasional collecting tubules in this case also.

Case 6 is likewise of considerable interest. The aspiration of large quantities of the gastric contents undoubtedly had contributed to the development of the severe alkalosis. It should be emphasized that this patient exhibited clinical evidence of renal impairment at the onset of therapy. Many of the renal changes were arteriosclerotic in origin.

Most of the glomeruli and approximately two thirds of the entire substance of each kidney appeared normal, however. The most striking observation was that of blue-staining material in numerous collecting tubules, some of which were obstructed and surrounded by an increased amount of interstitial fibrous tissue. Similar blue-staining material was visible also in the interstitial tissue itself, suggesting possibly a primary type of calcification in contrast to calcium precipitation. The parathyroid hyperplasia presumably was secondary to the renal insufficiency. The role of sulfanilamide toxicity in the genesis of the renal changes cannot be evaluated but, on the other hand, cannot be completely excluded.

*Group 3. Prolonged Ingestion of Massive Quantities of Alkali, with Death in the Course of Alkalosis.*

CASE 1.—A 59 year old man had for three years frequently required sodium bicarbonate for the relief of symptoms of ulcer. He was hospitalized for fourteen days, during which time he received a total of 609 Gm. of sodium bicarbonate, calcium carbonate and tricalate. Moderately severe chemical alkalosis was noted on the fifth and ninth days. One year and seven months later the patient's symptoms recurred, and he was instructed to take alkalis at hourly intervals and to aspirate his stomach each night. This program was continued for two months; the gastric content aspirated varied in amount from 3 to 6 ounces (93 to 186.5 cc.). Several days before hospitalization he began to vomit large amounts of gastric content, and just prior to entry he became disoriented and semicomatose. He was markedly dehydrated and emaciated; the blood pressure which formerly had been 140 systolic and 85 diastolic was now 80 systolic and 52 diastolic. Massive quantities of physiologic solution of sodium chloride, 5 per cent saline solution and 5 per cent dextrose solution were administered subcutaneously and intravenously. Two blood transfusions also were given. The patient failed to respond to this therapy; he became markedly cyanotic and died on the fifth hospital day. The serum carbon dioxide on the third day measured 42.6 millimols per liter; this diminished to 33 millimols twenty-four hours later. The blood chloride increased from 494 to 653 mg. per hundred cubic centimeters, while the nonprotein nitrogen varied between 140 and 146 mg. per hundred cubic centimeters.

Necropsy revealed the following conditions: a chronic duodenal ulcer near the pylorus; marked duodenal stenosis, exaggerated by kinking due to traction of the stomach on the hepatoduodenal ligament; gastric hypertrophy and dilatation; slight cardiac dilatation; marked bilateral hypostatic pulmonary hyperemia and edema; acute aspiration bronchopneumonia with beginning suppuration in the lower lobe of the right lung; fatty degeneration and hemosiderosis of the liver; extreme edema of the entire gastrointestinal tract; ascites; minimal senile arteriosclerosis.

The kidneys were dark purple-gray and firm; the perirenal fat was slightly adherent; the right kidney weighed 100 Gm. and the left 120 Gm. The capsules stripped easily, leaving very cyanotic surfaces with prominent stellate veins. The cortices were dark reddish purple; the cortical rays were indistinct; the cortices measured 6 mm. The corticomedullary area was very dark; the tips of the pyramids were quite pale and fibrous. Microscopic examination revealed a few foci of scarring under the capsules with occasional round cell infiltration. There was marked increase in interstitial fibrous tissue in the medullary pyra-

mids. Occasional scattered glomeruli were hyalinized, but the majority were practically normal. Bowman's spaces were well filled and nowhere were greatly dilated. Many of the tubules in the cortices had wide lumens and flat epithelium, and usually contained protein debris. A few tubules in the scarred areas of the kidneys were atrophic. Throughout the cortices single or several tubules contained epithelium with large pleomorphic nuclei. Occasional tubules contained pale to dark blue-staining granular masses. A few of these masses appeared to be obstructing the tubules, the epithelium of which was flat or focally missing. The obstructed tubules were located usually not far from the corticomedullary junction; none were recognized in Henle's loops. There was no marked sclerosis of the large or of the small arteries. Sudan IV stains demonstrated slight fatty degeneration of the tubular epithelium.

CASE 2.—A 49 year old man had experienced symptoms of ulcer for seven years. Large amounts of alkali had been taken during this time. His right kidney had been removed six years previously because of a tuberculous infection. Just prior to hospitalization the patient had taken huge amounts of sodium bicarbonate and had vomited frequently. Forty-two grams of calcium carbonate was given during the first six days. The patient continued to vomit, and on the seventh day the serum carbon dioxide measured 57.9 millimols per liter and the  $p_H$  was 7.69. He became slightly disoriented and was given 1,000 cc. of physiologic solution of sodium chloride intravenously. On the eighth day the serum carbon dioxide was 49.2 millimols per liter and the  $p_H$  was 7.66; the blood urea nitrogen was 78 mg. per hundred cubic centimeters. Coma developed at this time, and the patient was given 400 cc. of physiologic solution of sodium chloride intravenously and 1,500 cc. subcutaneously. Muscular fibrillations appeared, and these were followed by a series of four convulsions. The blood pressure, which had been 160 systolic and 85 diastolic on entry, rose to 300 systolic and 125 diastolic and then gradually fell, first to 180 systolic and 110 diastolic and then to 95 systolic and 50 diastolic. Auricular fibrillation associated with severe cyanosis was noted at this time. The cardiac irregularity disappeared after the administration of digitalis. On the ninth day the serum carbon dioxide measured 43 millimols per liter, the  $p_H$  was 7.53, and the blood urea nitrogen was 90 mg. per hundred cubic centimeters. The blood pressure fluctuated from 125 to 135 systolic and from 80 to 85 diastolic. On the tenth day the serum carbon dioxide was 31.4 millimols per liter; the  $p_H$  was 7.50; the creatinine was 3.7 mg. and the blood urea nitrogen 98 mg. per hundred cubic centimeters. Death occurred on the tenth day as a result of cardiac failure. Several specimens of urine contained occasional red and white blood cells.

Necropsy revealed the following conditions: two chronic peptic ulcers and one healing superficial ulcer on the lesser curvature of the body of the stomach; cardiac dilatation, especially of the right chambers of the heart, with slight hypertrophy of the left ventricle; hypostatic hyperemia and edema of the lungs with acute bilateral bronchopneumonia; passive congestion of the liver with fatty changes; moderate generalized arteriosclerosis with sclerosis of the coronary arteries; focal myocarditis; encapsulated caseous and caseocalcareous tuberculosis of the right epididymus; healed calcified tubercles in the upper lobes of the left lung and in the left hilar lymph nodes and the peritracheal lymph nodes.

The remaining left kidney weighed 225 Gm. The capsule stripped easily, leaving a surface mottled with cyanotic and very pale areas; the cut surfaces

bulged. The cortex measured 8 mm. and was somewhat mottled with pale areas, in which the vascular markings were indistinct, and darker areas, where they were well preserved. The medulla was thinner than normal, and the tips of the medullary pyramids were fibrous. Microscopically, the glomeruli were within normal limits. The tubules were slightly dilated and contained much protein precipitate, occasional granular and hyaline casts, and leukocytes. A few collecting tubules contained a crystalline material staining faintly blue with hematoxylin and eosin, suggesting calcium. The epithelium of these tubules showed mild degenerative and regenerative changes. Surrounding these cortical tubules were scattered foci of round cell infiltration. The arterioles and larger-sized arteries showed some thickening.

CASE 3.—A 62 year old man had taken large amounts of sodium bicarbonate for many years for the relief of gastrointestinal distress, averaging 1 pound (373 Gm.) of alkali per week during the past two years. Sixteen months ago he had been hospitalized elsewhere for five months because of severe epigastric pain and vomiting. The symptoms persisted, and the patient entered the Albert Merritt Billings Hospital. His blood pressure was found to be 222 systolic and 116 diastolic. During the first six days he received 150 Gm. of sodium bicarbonate

TABLE 3.—*Acid-Base Balance in Case 3*

Hospital Day	Serum Cl, mM per Liter	Serum CO <sub>2</sub> , mM per Liter	Serum pH
4th.....	81.4	45.6	....
6th.....	70.0	57.1	7.50-7.58
7th.....	72.6	55.5	....
8th.....	78.0	58.8	....
9th.....	147.0 (?)	30.9	7.38

and calcium carbonate. On the seventh day he became drowsy and irrational and was given 1,000 cc. of physiologic solution of sodium chloride intravenously. The patient's condition grew progressively worse, and he lapsed into a coma on the ninth day. Solutions of 5 and 10 per cent dextrose in physiologic solution of sodium chloride were administered parenterally without avail, and the patient died in cardiac failure. Gastric aspirations during the first six days yielded from 700 to 1,000 cc. of gastric contents daily. Determinations of the acid-base balance (table 3) revealed severe alkalosis, which persisted until the ninth day. Renal function was not determined; one specimen of urine contained albumin.

Necropsy revealed a chronic benign ulcer in the prepyloric region of the stomach, acute pulmonary edema, acute distention of the right side of the heart, acute passive hyperemia of the liver and kidneys and moderate sclerosis of the abdominal aorta.

The kidneys were moderately enlarged and together weighed 350 Gm. They were of firm consistency. The capsules stripped easily, leaving smooth surfaces. The cortical markings were somewhat indistinct; the pyramids were cyanotic. The renal pelves and ureters were normal. Microscopic examination revealed many hyalinized glomeruli. Focal hemorrhage and thrombosis of the glomerular tufts were noted occasionally; these changes were not far advanced. There was considerable degeneration of the tubular epithelium associated with fatty degeneration. Rare tubules were lined by hyperplastic epithelium; a few eosinophilic and hyaline casts were seen. There was moderate scarring of the interstitial



tissue in the cortex, especially just below the capsule. No precipitation of calcium was observed. Some of the arterioles were markedly hyalinized, while others appeared relatively normal. The large arteries showed only moderate sclerosis.

CASE 4.—A 37 year old woman entered the hospital Jan. 18, 1935 with a history of distress from ulcers for five years. Alkalis had been taken occasionally during this time. The blood pressure was elevated, ranging from 154 to 170 systolic and from 88 to 110 diastolic. Seven hundred and forty-three grams of calcium carbonate and 826 Gm. of sodium bicarbonate were given in twenty-five days. Nine examinations of the urine gave negative results; the maximum specific gravity was 1.021. The blood urea nitrogen measured 11.3 mg. per hundred cubic centimeters, and the urea clearance was 65 per cent of average normal. Ulcer therapy was continued faithfully for the next year, and the patient remained fairly well except for frequent episodes of vomiting. She was readmitted April 10, 1936 because of massive hemorrhage. The treatment included parenteral adminis-

TABLE 4.—*Acid-Base Balance and Urea Clearance in Case 4.*

Hospital Day	Serum Cl, mM per Liter	Serum CO <sub>2</sub> , mM per Liter	pH	Blood Urea N, Mg. per 100 Cc.	Urea Clear- ance, % Av. Normal	Ca, Mg. per 100 Cc.	P, Mg. per 100 Cc.
7th.....	....	62.1	7.65	....	..	...	...
8th.....	....	50.5	7.58	....	..	...	...
10th.....	....	36.0	7.53	....	..	...	...
14th.....	....	36.6	7.52	....	..	...	...
17th.....	83.7	37.4	7.52	....	..	...	...
21st.....	101.5	35.4	7.50	28.2	..	...	...
22d.....	....	....	....	25.4	22	...	...
28th.....	99.1	32.6	7.49	....	..	6.2	...
30th.....	....	....	....	....	..	7.5*	...
32d.....	....	....	....	95.0	52	...	...
35th.....	79.0	46.0	7.49	....	..	9.8	...
49th.....	....	....	....	....	..	9.9	3.9

\* Plasma protein amounted to 6.4 Gm. per hundred cubic centimeters: albumin/globulin = 5.12/1.28 = 4.00.

tration of fluids and continuous aspiration of the stomach each night. Six hundred and seventy-four grams of calcium carbonate, 480 Gm. of sodium bicarbonate and 960 Gm. of tricalstate were given in forty-five days. Because of pyloric obstruction, gastroduodenostomy was performed on the thirty-ninth day, from which the patient made an uneventful recovery. Studies of the acid-base balance revealed severe alkalosis (table 4). On the twenty-seventh day a flexor spasm of both the fore-arms and the hands and numbness of the face developed; the Chvostek sign was positive. Three thousand cubic centimeters of 5 per cent dextrose in physiologic solution of sodium chloride was given parenterally, and the patient recovered promptly. It will be noted that the acid-base balance at this time was practically normal. The serum calcium was markedly lowered but returned to normal within seven days. Two of ten specimens of the urine contained a trace of albumin; the specific gravity ranged from 1.005 to 1.022. The patient took alkalis regularly for the next two years but entered the hospital a third time Dec. 21, 1937, thirty-five months after the initial visit, because of recurrent hemorrhage. She had been vomiting for nine days before admission. Three hundred and thirty grams of calcium carbonate and 900 Gm. of sodium bicarbonate were given in thirty-one days. The acid-base balance on the third day was normal; the blood urea nitrogen

measured 11.1 mg. per hundred cubic centimeters, and the urea clearance was 42 per cent of average normal. Eighteen days later there was a slight rise in the serum carbon dioxide (32 millimols per liter) and  $p_H$  (7.56); the blood urea nitrogen now measured 23.6 mg. per hundred cubic centimeters and the urea clearance 22 per cent. On the twenty-ninth day, however, the acid-base balance was normal; the blood urea nitrogen was 12.1 mg. per hundred cubic centimeters and the urea clearance 60 per cent. Seven examinations of the urine gave negative results; the specific gravity ranged from 1.008 to 1.018. Alkali therapy was continued subsequently for almost two years. The patient vomited frequently.

The fourth admission to the hospital occurred Nov. 5, 1940, five years and ten months after the original entry. The blood pressure at this time was 170 systolic and 94 diastolic. Wangensteen aspiration of the stomach was carried out for ten hours each day. Three thousand cubic centimeters of 5 per cent dextrose in distilled water was administered parenterally daily. This program was discontinued after five days, and on the seventh day salt was added gradually to the regimen. Eighty-eight grams of calcium carbonate was given in the last

TABLE 5.—*Acid-Base Balance in Case 4 (Subsequent Visit)*

Hospital Day.	Serum Cl, mM per Liter	Serum CO <sub>2</sub> , mM per Liter	$p_H$	Blood Urea N, Mg. per 100 Cc.	Serum Ca, Mg. per 100 Cc.
2d.....	95.9	39.4	7.54	9.6	...
3d.....	....	....	....	21.5	...
5th.....	87.6	35.6	7.58	....	...
6th.....	86.8	35.1	7.61	....	...
7th.....	80.0	41.2	7.60	....	...
10th.....	56.7	41.3	7.47	18.6	...
11th.....	64.3	37.4	7.67	....	...
12th.....	80.1	....	....	9.4	...
14th.....	86.2	37.2	7.53	6.1	8.9
15th.....	....	....	....	8.8	...
16th.....	92.6	37.0	7.52	....	...

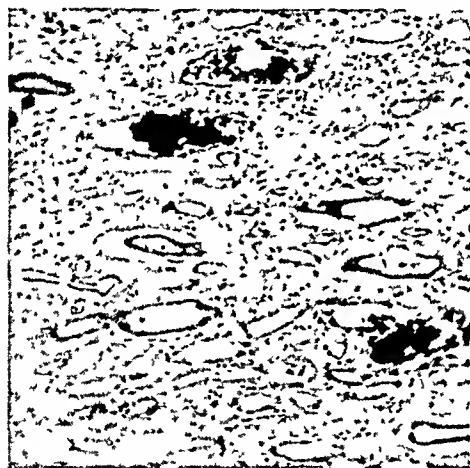
four days of this hospital period. A trace of albumin was noted in one of six specimens of the urine; the specific gravity ranged from 1.011 to 1.014. Numerous studies of the acid-base balance were made as shown in table 5.

The patient vomited frequently during the next month. She was readmitted for the last time Dec. 28, 1940 in a semicomatose condition. The reflexes were hyperactive, and there were coarse fibrillary muscular twitchings. The serum chloride measured 71 millimols per liter; the serum carbon dioxide, 48.5 millimols per liter. The  $p_H$  was 7.70. Fifteen hundred cubic centimeters of physiologic solution of sodium chloride was given parenterally to no avail. The patient had clonic convulsions and died several hours later.

Necropsy revealed an acute gastric ulcer with high grade stenosis of the pylorus, a chronic duodenal ulcer with high grade stenosis of an old gastro-duodenostomy and perforation into the head of the pancreas, recent hemorrhage throughout the head of the pancreas and recent hemorrhage and edema of the lungs. Two parathyroid glands were normal grossly and histologically.

The left kidney weighed 100 Gm. The capsule stripped with moderate difficulty, leaving a diffusely granular pale surface. The cortex measured 4 to 6 mm. and the medulla 14 to 15 mm. The cortical rays were distinct; the pyramids were slightly hyperemic. The renal pelvis was normal; there was one recent subcapsular hemorrhage. The right kidney weighed 112 Gm. and was essentially similar in

appearance to the left kidney. Microscopic examination revealed an increase in fibrous tissue and a few fine cortical scars with foci of round cells near the capsule. A few glomeruli were completely hyalinized, and many others showed some degree of sclerosis, chiefly due to an increase in hyaline ground substance. In occasional glomeruli there was marked thickening of the basement membranes. The tubules varied considerably in appearance. Some were widened and lined by flat cuboidal cells; others had epithelium with hyperchromatic nuclei of varying size and form, and mitotic figures were seen occasionally, indicating active cell proliferation. There was extensive desquamation of the epithelium in some of the distal collecting tubules, and the lumens often were completely obstructed by loose epithelial cells and granular protein debris. Hyaline casts were few. Many collecting tubules were filled with coarse blue-staining or almost colorless granular masses which stained black with von Kossa's stain (fig.), indicating that they were composed of calcium. The epithelium of these tubules was necrotic. Foci of interstitial inflammation were noted surrounding the calcium-filled tubules. Some arterioles had thickened hyaline walls, but many of the vessels showed little change. The Gomori<sup>8</sup> stain revealed a markedly decreased alkaline phosphatase content of the kidney.



Photomicrograph demonstrating deposition of calcium in renal collecting tubules in case 4, group 3.

Although the kidneys in case 1 showed acute and chronic degenerative changes, most of the renal parenchyma was normal. Calcium precipitate was present in occasional collecting tubules. A few tubules were completely obstructed by these masses, and their epithelium presented mild degenerative and regenerative changes. Similar but less marked findings were noted in case 2, in which the alkalosis also had been severe. The kidneys in case 3 showed the typical findings of nephrosclerosis secondary to malignant hypertension. The changes in the tubules are attributable to the passive congestion. None of the findings could be related to the alkali therapy or to alkalosis. Case 4 was of great interest not only because the patient had taken large

8. Gomori, G.: *J. Cell. & Comp. Physiol.* **17**:71, 1941.

quantities of alkali for six years but also because there had been prolonged and excessive depletion of chloride through vomiting and gastric aspiration. She had experienced one episode of mild alkalosis and three bouts of severe alkalosis. Although considerable renal damage was visible, much of the parenchyma appeared fairly normal. Many of the changes, i. e., hyalinization of the glomeruli, tubular atrophy, increase of fibrous tissue and arteriolar thickening, are attributable to the hypertension which had been present. The precipitation of calcium in the lumens of the collecting tubules with obstruction of some of these tubules was more extensive in this case than in any other of the entire series. The histologic alterations resemble those of the interstitial pyelonephritis seen in patients not given alkalis; this possibility must be considered even in the absence of a suggestive history.

It is important to note that all patients of this group had vomited excessively and that all but 1 had had hypertension.

#### COMMENT

The most interesting histologic change in the kidney noted in this study was the calcium precipitate in the collecting tubules. Some of these tubules were obstructed by the calcium masses, and their epithelium showed both degenerative and regenerative changes. This finding was present in the kidneys of 3 patients who had previously experienced alkalosis but who did not die during the acid-base disturbance and in those of 3 patients in whom alkalosis was present at the time of death. All 6 patients had lost excessive amounts of chloride either by vomiting or by gastric aspiration.

The precipitation of calcium in the collecting tubules of the kidney is of considerable interest, although in the present study it did not appear to be of sufficient severity to destroy the function of more than relatively few nephrons. It is not a specific complication of "alkali alkalosis." It was first described by Nazari<sup>9</sup> in 1904 in 2 patients with vomiting secondary to pyloric stenosis. Many writers<sup>10</sup> subsequently emphasized its occurrence in persons with obstruction of the pylorus and of the upper part of the intestine, none of whom had received alkalis. It has been observed also in the kidneys of dogs after prolonged and severe

9. Nazari, A.: *Policlinico (sez. med.)* **11**:146, 1904.

10. (a) Brown, G.; Eusterman, G. B.; Hartman, H. R., and Rowntree, L. G.: *Arch. Int. Med.* **32**:425, 1923. (b) Zeman, F. D.; Friedman, W., and Mann, L. T.: *Proc. New York Path. Soc.* **24**:41, 1924. (c) Borst, J. G. G.: *Ztschr. f. klin. Med.* **117**:55, 1931. (d) Cooke, A. M.: *Quart. J. Med.* **2**:539, 1933. (e) Butler, A. M.; Wilson, J. L., and Farber, S.: *J. Pediat.* **8**:489, 1936. (f) Nicol, B. M.: *Quart. J. Med.* **9**:91, 1940. (g) Martz, H.: *Arch. Int. Med.* **65**:375, 1940.

depletion of chloride.<sup>11</sup> Similar changes have been noted by us after alkalosis secondary to continuous aspiration of gastric contents, as in the following case in which alkali had never been prescribed.

A 50 year old man entered the hospital with peritonitis following rupture of a gangrenous appendix. Because of abdominal distention, he was treated by continuous Wangenstein aspiration. Alkalosis developed despite the administration of saline solution, and the patient subsequently died of cardiac failure and bronchopneumonia. The blood chloride which had decreased to 214 mg. per hundred cubic centimeters rose to 436 mg. after the use of saline solutions; the carbon dioxide measured 28.3 millimols per liter, while the  $p_H$  varied from 7.54 to 7.59. The blood urea nitrogen was extremely high, measuring 231 and 256 mg. per hundred cubic centimeters; the blood creatinine was elevated to an unusual degree, measuring 6.75 and 7.0 mg. per hundred cubic centimeters. Microscopic examination of the kidneys revealed the presence of blue-staining material, probably calcium, in many of the collecting tubules. The epithelium of these tubules showed degenerative changes. There were numerous foci of interstitial inflammation; considerable protein debris as well as numerous casts were present in the lumens of the tubules. The histologic changes were identical with those observed in the 6 patients previously described.

The mechanism of this condition is not entirely clear. Calcium may be deposited in necrotic cells. The renal parenchyma often is normal, however, and the process may occur too rapidly for extensive necrosis to take place. Kerpel-Fronius and Martyn<sup>12</sup> produced salt deficiency in cats by ligation of the pylorus and noted calcium precipitation only in those animals in which both dehydration and alkalosis occurred; in the cats in which the development of alkalosis was prevented, only fatty degeneration of the tubules was observed. Hatano<sup>13</sup> was able to prevent this complication during experimental hypochloremia by the intravenous injection of sodium chloride, further emphasizing the importance of depletion of chloride in this process. The deposition of calcium in the kidney after alkalosis is due apparently to an alteration in the physical-chemical state of the urine resulting presumably in the precipitation of calcium phosphate and calcium carbonate. Enzymes, such as alkaline phosphatase, probably play no role in its development.<sup>14</sup> Zeman and associates<sup>10b</sup> studied the process in cats in which alkalosis was allowed to develop after ligation of the pylorus. The presence of calcium in the kidney was noted within forty-eight hours. Gömöri and Sarmai<sup>15</sup> later confirmed this observation and demonstrated that it was a reversible process, the calcium disappearing rapidly after removal of the pyloric obstruction.

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12. Kerpel-Fronius and Martyn, cited by Gömöri, P.; Podhradszky, L., and Kring, J.: *Acta med. Scandinav.* **102**:591, 1939.

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15. Gömöri, P., and Sarmai, E.: *Klin. Wchnschr.* **46**:1, 1939.

## SUMMARY

No special histologic changes were noted in the glomeruli of the kidneys of 14 patients after prolonged alkali therapy with and without alkalosis. The epithelium of the collecting tubules in the kidneys of 6 patients showed degenerative and regenerative changes. There was precipitation of calcium in the lumens of these tubules. The tubules which did not contain calcium usually appeared normal. Three of these 6 patients had previously experienced alkalosis but did not die during the acid-base disturbance; alkalosis was present at the time of death in the other 3. All 6 patients had lost excessive amounts of chloride by vomiting or therapeutic aspiration. Similar changes were observed in a patient not receiving alkali but in whom severe hypochloremia and alkalosis developed as a result of gastric aspiration.

## CONCLUSION

The long-continued administration of alkali in man with or without alkalosis does not lead to significant anatomic change in the kidneys attributable to alkali.

# AMYLOID

## III. THE PROPERTIES OF AMYLOID DEPOSITS OCCURRING IN SEVERAL SPECIES UNDER DIVERSE CONDITIONS

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In a recent study the end point of the alkaline solubility of the amyloid occurring in tissues of patients with chronic pulmonary tuberculosis was determined.<sup>1</sup> By taking advantage of the sharpness of this end point, solutions of amyloid that were not unduly contaminated with other soluble tissue components were obtained. From these solutions three fractions were isolated. The principal fraction was found to have the physical properties and elementary chemical constituents of a protein.<sup>2</sup> One minor fraction was identified as a sulfate-bearing polysaccharide.<sup>2a</sup> A second minor fraction has not been identified, although its physical behavior indicates that it is a protein.<sup>1</sup>

The designated studies were restricted to amyloid occurring in one species during the course of one disease. The present studies are more comprehensive and represent an inquiry into the question of variation in the composition of amyloid. This inquiry is concerned principally with relations between the solubilities of amyloid, the staining properties of the matrix and the apparent causes of amyloid disease in man, horses and rabbits.

### MATERIAL AND METHODS

*Species.*—A study was made of tissues of man, horses, rabbits and mice.

*Abnormal Conditions Serving as Apparent Causes of Amyloid Disease in This Study.*—In this study amyloid disease was never encountered in an otherwise normal subject. For convenience the abnormal conditions which preceded and accompanied the development of the disease were regarded as noninfectious and infectious, singly or in combination.

The noninfectious abnormal conditions were of two types. One type was produced experimentally by successive injections of several antigens. The second type was produced by the disease known as plasma cell myeloma.

Horses, rabbits and mice were used in experiments in which antigens were injected. Diphtheria toxin, tetanus toxin, plain streptococcus toxin, scarlet fever

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From the Department of Pathology of Cornell University Medical College.

1. Hass, G. M., and Schulz, R. Z.: Arch. Path. **30**:240, 1940

2. (a) Hass, G. M.: Arch. Path. **34**:92, 1942. (b) Hass.<sup>1</sup>

TABLE 1.—*Relations Between the Method of Immunization of Horses, the Duration of Immunization, the Formation of Amyloid in Organs and the Iodine Reaction*

No.	Equine Organ Examined	Amount of Amyloid	Iodine Test	Immunization Data
9	Spleen Liver Kidney	0 + 0	.. ± ..	4/23/36 to 6/ 6/39 7/23/36 to 1/30/40 Agar mass Scarlet fever streptococcus toxin
10	Spleen Liver Kidney	+ + 0	± ± ..	5/26/36 to 2/ 4/38 8/ 6/36 to 10/10/38 10/10/38 to 10/ 4/39 10/ 4/39 to 1/30/40 Agar mass Plain streptococcus toxin Scarlet fever streptococcus toxin Plain streptococcus toxin
11	Spleen Liver Kidney	++ ++ 0	++++ ++++ ..	8/18/38 to 3/ 6/40 ..... Tetanus toxin Tetanus toxin + 0.5% alum
12	Spleen Liver Kidney	+ + 0	++ ++ ..	3/ 6/37 to 2/ 7/38 2/14/38 to 11/ 2/38 11/ 2/38 to 3/11/40 Plain streptococcus toxin Diphtheria toxin Pneumococcus type XVIII (killed)
13	Spleen Liver Kidney	++ +++ 0	++++ ++++ ..	10/24/38 to 3/13/40 ..... Tetanus toxin Tetanus toxin + 0.5% alum
14	Spleen Liver Kidney	0 + 0	.. ± ..	11/ 9/36 to 4/ 3/39 ..... 7/ 6/38 to 12/ 7/39 Diphtheria toxin Diphtheria toxin + 0.3% CaCl <sub>2</sub> Pneumococcus type I (killed)
15	Spleen Liver Kidney	++ ++ 0	+ + ..	11/ 9/36 to 11/ 2/38 11/ 2/38 to 8/ 2/39 10/ 9/39 to 4/13/40 Diphtheria toxin Pneumococcus type IV (killed) Tetanus toxin
16	Spleen Liver Kidney	+++ ++++ ..	± ± ..	7/ 4/28 to 5/16/40 ..... ..... Tetanus toxin Tetanus toxin + 0.3% CaCl <sub>2</sub> Tetanus toxin + 0.5% alum
17	Spleen Liver Kidney	+ + 0	— — ..	5/18/37 to 3/15/38 ..... 3/15/39 to 1/11/40 Diphtheria toxin Diphtheria toxin + 0.3% CaCl <sub>2</sub> Plain streptococcus toxin; scarlet fever streptococcus toxin
18	Spleen Liver Kidney	++ +++ 0	+++ ++ ..	7/13/37 to 1/ 5/39 4/23/38 to 4/25/40 ..... Diphtheria toxin Tetanus toxin Tetanus toxin + 0.3% CaCl <sub>2</sub> tetanus toxin + 0.5% alum
19	Spleen Liver Kidney	0 0 0	.. .. ..	4/ 7/37 to 5/31/38 5/31/38 to 3/10/40 ..... Scarlet fever streptococcus toxin Tetanus toxin Tetanus toxin + 0.5% alum
20	Spleen Liver Kidney	0 0 0	.. .. ..	4/27/37 to 3/15/40 Pneumococcus type IV (killed)
21	Spleen Liver Kidney	0 0 0	.. .. ..	3/10/37 to 3/14/40 Pneumococcus type IV (killed)
22	Spleen Liver Kidney	0 0 0	.. .. ..	6/ 2/36 to 4/ 5/40 Pneumococcus type I (killed)
23	Spleen Liver Kidney	0 0 0	.. .. ..	5/ 2/38 to 3/18/40 Pneumococcus type IV (killed)
24	Spleen Liver Kidney	0 0 0	.. .. ..	8/ 1/36 to 8/20/36 8/20/36 to 3/31/40 Pneumococcus type I (killed) Pneumococcus type VII (killed)
25	Spleen Liver Kidney	0 0 0	.. .. ..	2/ 1/38 to 4/16/40 Pneumococcus type XVIII (killed)
26	Spleen Liver Kidney	0 0 0	.. .. ..	11/ 9/36 to 5/17/37 ..... 5/17/37 to 5/ 1/40 Diphtheria toxin Diphtheria toxin + 0.3% CaCl <sub>2</sub> Pneumococcus type I (killed)
27	Spleen Liver Kidney	0 0 0	.. .. ..	7/17/39 to 1/10/40 3/18/40 to 5/28/40 Plain streptococcus toxin Diphtheria toxin + 0.5% CaCl <sub>2</sub>
28	Spleen Liver Kidney	0 0 0	.. .. ..	9/ 7/38 to 11/25/38 11/25/38 to 6/25/40 Diphtheria toxin Diphtheria toxin + 0.5% CaCl <sub>2</sub>
29	Spleen Liver Kidney	0 0 0	.. .. ..	20 years old ..... No immunization



streptococcus toxin and several types of heat-killed pneumococci were injected singly or in diverse combinations into 20 horses. The antigen or antigens administered to each horse and the periods of active immunization are listed in table 1.

Eight rabbits were given injections of several types of heat-killed pneumococci. Except for occasional five day intervals of rest, the injections were made intravenously three or four times each week over a period of four months.

Details of the methods used in the immunization of the horses and the rabbits need not be presented here. Suffice it to state that the animals were used for the production of immune serums under the direction of Dr. Jules Freund at the Research and Antitoxin Laboratory, Otisville, N. Y.<sup>3</sup>

Ninety-six young white mice were given injections of sodium caseinate. A fresh 5 per cent aqueous solution of this antigen was prepared every ten days by addition of the calculated quantity of sodium hydroxide to purified casein. The solution was sterilized by filtration and preserved at 5 C. in sterile tubes. Each mouse was given 0.25 cc. of the solution subcutaneously every five days. The experiment was terminated at the end of eight months. Forty-two mice lived throughout the experiment. Twenty-three mice which died during the experiment were likewise available for examination.

The second type of noninfectious condition which served as an apparent cause of amyloidosis was the disease plasma cell myeloma. A brief record of the only case in which material was obtained for study is as follows:

A man 39 years of age had been in good health until 1935, when he had an attack of acute arthritis. This subsided within a few weeks. In 1938 arthritis recurred and persisted. Many small and large joints were swollen and painful. As the arthritis progressed, limitation of motion of the joints increased, and firm tumor-like masses gradually developed in the subcutaneous tissues, especially around the larger joints. Laboratory tests disclosed severe progressive anemia, impairment of renal function and large quantities of protein in the urine. Several tests for Bence Jones protein in the urine showed none. The blood serum protein was always high. The maximum value was 9.5 Gm., with a serum globulin value of 7.3 Gm. and a serum albumin value of 2.2 Gm. per hundred cubic centimeters. The serum calcium value varied from 12.4 to 13.7 mg. and the serum phosphorus value from 3.6 to 4.9 mg. per hundred cubic centimeters.

Roentgenograms showed general osteoporosis with disseminated cystic areas of destruction of bone.

Biopsy of the tongue and of a subcutaneous nodule revealed masses of afibrillar homogeneous tissue, recognized as deposits of amyloid.

A differential count of cells in a specimen of the sternal marrow revealed a high percentage of plasma cells and led to a diagnosis of plasma cell myeloma.

The patient, aggravated by dysphagia and diminishing mobility of the tongue, gradually became weaker and died in September 1939, four years after the onset of symptoms of arthritis.

The postmortem examination established the diagnoses of plasma cell myeloma and amyloid disease. The kidneys, which contained large deposits of amyloid in the glomeruli, several subcutaneous periarticular masses of tissue which were composed almost exclusively of amyloid, and large blocks of skeletal muscle in which muscle fibers were replaced by amyloid, were preserved in the fresh state for study.

In the present investigation the only infectious disease which served as an apparent cause of amyloidosis was tuberculosis. Tissues were obtained from two

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3. The tissues of horses and rabbits which had been immunized with pneumococci were received from the Research and Antitoxin Laboratory of the Department of Health of the City of New York, in Otisville, N. Y.

species, man and the rabbit. All the human subjects of amyloid disease, 21 in number, had chronic pulmonary tuberculosis due to the human tubercle bacillus. The fresh tissues were obtained post mortem through Dr. Reuben Schulz, of the

TABLE 2.—*Relations Between the Tuberculous Infection in Rabbits, the Duration of the Disease, the Superimposed Immunization, the Occurrence of Amyloid Disease and the Iodine Reaction of Amyloid Deposits*

No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	Infection	Immunization
1	Spleen Liver Kidney	++++ + +++	+ + +	Bovine tubercle bacillus (7 months)	None
2	Spleen Liver Kidney	++ 0 ++++	++ ++ ++	Bovine tubercle bacillus (10 months)	None
3	Spleen Liver Kidney	++ 0 +++	++ ++ ++	Bovine tubercle bacillus (10 months)	None
4	Spleen Liver Kidney	+++ + +++	++++ +++ +++	Human tubercle bacillus (12 months)	None
5	Spleen Liver Kidney	+++ ++ ++	— — —	Bovine tubercle bacillus (7 months)	Heat-killed tubercle bacilli in liquid petrolatum
6	Spleen Liver Kidney	+++ ++ ++	— — +++	Bovine tubercle bacillus (11 months)	Heat-killed tubercle bacilli in liquid petrolatum
7	Spleen Liver Kidney	++++ ++ +++	— — +	Bovine tubercle bacillus (11 months)	Heat-killed tubercle bacilli in liquid petrolatum
8	Spleen Liver Kidney	++++ +++ ++++	++ — ++	Bovine tubercle bacillus (12 months)	Heat-killed tubercle bacilli in liquid petrolatum
9	Spleen Liver Kidney	++++ ++ +++	..	Bovine tubercle bacillus (3 months)	6 cc. tuberculin each week for 12 weeks during infection
10	Spleen Liver Kidney	+++ ++ ++++	..	Bovine tubercle bacillus (3 months)	6 cc. tuberculin each week for 12 weeks during infection
11	Spleen Liver Kidney	+++ ++ +++	..	Bovine tubercle bacillus (3 months)	6 cc. tuberculin each week for 12 weeks during infection
12	Spleen Liver Kidney	++++ ++ ++	..	Bovine tubercle bacillus (3 months)	6 cc. tuberculin each week for 12 weeks during infection
13	Spleen Liver Kidney	0 0 0	..	Bovine tubercle bacillus (3 months)	None
14	Spleen Liver Kidney	0 0 0	..	Bovine tubercle bacillus (3 months)	None
15	Spleen Liver Kidney	0 0 0	..	Bovine tubercle bacillus (3 months)	None
16	Spleen Liver Kidney	0 0 0	..	Bovine tubercle bacillus (3 months)	None

Middlesex Hospital, Cambridge, Mass., and Dr. Oscar Auerbach, of the Sea View Hospital, Staten Island, N. Y.

There were three groups of rabbits with tuberculosis and amyloid disease. These are listed in table 2. In the first group there were 4 animals. Three of these were infected by intravenous injections of bovine tubercle bacilli and the fourth by an intravenous injection of human tubercle bacilli. They lived for periods varying from seven to twelve months after onset of the infection. In the second

group of rabbits the bovine tuberculous infection was complicated by a course of immunization with heat-killed bovine tubercle bacilli suspended in liquid petrolatum. There were 4 rabbits in this series. They survived for periods varying from seven to twelve months. In the third group of rabbits the bovine tuberculous infection was complicated by weekly injections of 6 cc. of old tuberculin. In this experiment there were 8 animals. Four of these received injections of tuberculin during the three months' course of the tuberculous infection. The remainder, which served as controls, received the same dosage of tubercle bacilli but no injections of tuberculin. Tissues from these animals were obtained through Dr. Eugene Opie and his collaborators, Drs. Robert Huntington, Richard Linton and Newton Krumdieck.

#### EXAMINATION OF TISSUES

The study of amyloid deposits was routinely made on the spleen, the liver and the kidney. In the instance of the patient with plasma cell myeloma, the study was restricted to the kidney and to massive deposits of amyloid in the muscular and periarticular tissues. All tissues except those from the controlled series of tuberculous rabbits given tuberculin were available in the fresh state.

The fresh tissues were divided into two parts. One part was used for the study of the solubilities and morphologic properties of the amyloid. The second part was preserved in 4 per cent solution of formaldehyde for routine microscopic study in permanent sections.

The study of the solubilities and morphologic properties of amyloid was conducted as follows: Fresh blocks of tissue were cut into sections with a freezing microtome set at 20 to 25 microns. Sections of each organ or tissue were divided into four groups. One group was stained with compound solution of iodine U. S. P. (Lugol's solution) diluted so as to contain 0.1 per cent iodine, a second group with a 0.01 per cent aqueous solution of crystal violet and a third group with a 2 per cent aqueous solution of congo red. The fourth group was set aside for determinations of solubility. After making an immediate microscopic estimate of the amount of amyloid and of the quality of the staining reactions of the deposits, the residual unstained fresh sections were so divided that several sections were available for extraction with each of the following mediums: unbuffered aqueous fiftieth, seventy-fifth and hundredth normal solutions of sodium hydroxide, fifth normal solution of barium hydroxide, saturated solution of calcium hydroxide, saturated solution of strontium hydroxide and phosphate buffer solutions with  $p_H$  values of 10, 11, 11.2, 11.6 and 12. After sections were put in each solution, the flasks were sealed and kept at 5 C. At the end of twelve hours, three sections were removed from each flask, neutralized by washing successively in aqueous tenth-normal acetic acid and water and then stained with the standard solutions of iodine, crystal violet and congo red. The stained sections were studied microscopically at once. Estimates were made of the effects of the various solvents on the quantity and the staining properties of the amyloid. This procedure was repeated a second time after a total extraction period of twenty-four hours.

In addition to these routine studies there were several random experiments which were devised to test the action of buffer solutions of  $p_H$  values 1 to 10 on the deposits of amyloid in the different species.

#### RESULTS

All horses remained in good health during the courses of immunization by repeated injections of one or more noninfectious antigens. They

were killed while still in good health, and no evidence of infection was found at postmortem examination, though most of the horses had intestinal parasites. Amyloid deposits as recorded in table 1 were found. Inasmuch as most horses had been given injections of more than one type of antigen over different periods, as recorded in table 1, an exact analysis of the relations between the antigen or antigens and the characteristics of the amyloid deposits was impossible. Nevertheless, it was clear that all antigens were not equally effective in the production of amyloid disease. Tetanus toxin was most effective; the other antigens may be listed in order of decreasing effectiveness as follows: streptococcus toxins, diphtheria toxin and heat-killed pneumococci. None of the four types of pneumococci when used alone was capable of acting as an apparent cause of amyloidosis. The type of antigen was a more important factor in the production of amyloid disease in horses than the duration of the course of immunization beyond a minimum interval of time. Furthermore, there was no evidence that the increment in the amount of amyloid was principally dependent on prolongation of the minimum interval of time. For instance, in horse 13 severe amyloid disease developed during immunization with tetanus toxin over a period of eighteen months. Horse 19, treated in a similar way for twenty-one months, did not acquire amyloid disease, and horse 16, intensively immunized with tetanus toxin for twelve years, acquired but little more amyloid than the horse that had received the toxin for only eighteen months.

The only noninfectious antigens injected into normal rabbits were killed pneumococci. As in horses, these antigens were not effective in producing amyloid disease.

The only noninfectious antigen injected into mice was sodium caseinate. Despite prolongation of the period of immunization to eight months, the mice did not acquire amyloid disease.

So far as could be determined, the single case of plasma cell myeloma was not complicated by infection or stimulation of immune mechanisms by a noninfectious antigen. Nevertheless, as is occasionally observed in this disease, there was widespread amyloidosis. The distribution of the amyloid was unusual. The deposits were restricted principally to the walls of small arterioles, including those of renal glomeruli, to striated skeletal muscle and to periarticular or subcutaneous connective tissue. The intramuscular and periarticular deposits were especially abundant and often grossly resembled masses of hyaline cartilage. Microscopically, these masses were composed principally of homogeneous afibrillar tissue in which scattered fibrocytes were embedded.

In the present studies the tubercle bacillus was the only infectious agent which seemed to be responsible for the development of amyloid disease. This does not necessarily mean that the tubercle bacillus was the only infectious agent in the tissues. Other bacteria were often

present, especially in the chronic pulmonary lesions of several human beings with amyloid disease, but in the rabbits with experimental bovine tuberculosis superimposed nontuberculous infections were of little significance.

All the tuberculous infections were severe and prolonged, but in several human beings only one lobe of a lung was involved, and in 2 rabbits the tuberculous lesions were not conspicuous. The minimum duration of active tuberculosis was two years among the human subjects and seven months among the rabbits. The majority of the human subjects had had the disease over a period of many years. The incidence of amyloid disease in tuberculous rabbits increased rapidly in the second six month period of infection. Indeed, studies not reported here have revealed that in a large series of rabbits about 3 of every 4 animals had amyloid disease within fifteen months after onset of infection. Despite these findings, no consistent relation between the degree or the duration of tuberculosis and the quantity of amyloid in the tissue was found.

When rabbits with experimental bovine tuberculosis were given injections of an antigen, as recorded in table 2, amyloid deposits were often very prominent. We are not prepared to say whether the use of killed tubercle bacilli suspended in liquid petrolatum had any influence on the development of amyloid disease. However, it was clear that repeated injections of old tuberculin into animals during the course of a tuberculous infection led to the development of severe amyloid disease in three months. The control infected rabbits at the end of three months had about the same degree of tuberculosis but no evidence of amyloid disease. The injection of tuberculin into infected animals, therefore, not only hastened the onset of amyloid disease but also led to the rapid accumulation of a large quantity of amyloid in the tissues.

Though the results indicate that there is a species susceptibility to amyloidosis, adequately controlled studies will be required before the susceptibilities can be properly defined. Until such studies are undertaken, the following order of diminishing species susceptibility to amyloid disease may be proposed: rabbit, horse, man and mouse.

The quantitative distribution of amyloid in the spleen, the liver and the kidney differed among the several species. These differences are shown in tables 1, 2 and 8. In horses, amyloid deposits were more common in the spleen than in the liver, and no amyloid was found in the renal glomeruli. In rabbits the spleen always contained a larger amount of amyloid than the liver. Frequently, almost the entire structure of the spleen, especially the extrafollicular structure, was replaced by amyloid, while massive deposits were never found in the liver. As a rule, whenever amyloid was found in the spleen, it was likewise present in renal glomeruli and often in large amounts. In these instances the kidneys were enlarged, pale and usually the site of degenerative changes similar

to those described in amyloid nephrosis of human beings. When these renal changes were conspicuous, the impression was gained that they were responsible for the death of several animals, especially those with minimal tuberculosis. The distribution of amyloid in organs of the human subjects with tuberculosis, though not tabulated fully, was the same as that which is generally known. In severe disease, the deposits of amyloid were more voluminous in the liver than in the spleen, and the renal glomeruli were frequently normal even when 20 to 30 per cent of the spleen and the liver were replaced by amyloid. The susceptibility

TABLE 3.—*The Range of Solubility of Equine Amyloid in Phosphate Buffer Solutions at 5 C.*

No.	Equine Organ Examined	Amount of Amyloid	Iodine Test	Solubility of Amyloid at 5 C.						
				pH 10 (24 Hr.)	pH 11 (12 Hr.)	pH 11 (24 Hr.)	pH 11.6 (12 Hr.)	pH 11.6 (24 Hr.)	pH 12 (12 Hr.)	pH 12 (24 Hr.)
9	Spleen	0								
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
10	Spleen	+	±	0	++++	++++	++++	++++	++++	++++
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
11	Spleen	++	++++	0	+	++	+++	++++	++++	++++
	Liver	++	++++	0	+	++	+++	++++	++++	++++
	Kidney	0								
12	Spleen	+	++	0	++++	++++	++++	++++	++++	++++
	Liver	+	++	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
13	Spleen	++	++++	0	+++	++++	++++	++++	++++	++++
	Liver	+++	++++	0	+++	++++	++++	++++	++++	++++
	Kidney	0								
14	Spleen	0								
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
15	Spleen	++	+	0	++++	++++	++++	++++	++++	++++
	Liver	++	+	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
16	Spleen	+++	±	0	+	+	++	++	++++	++++
	Liver	++++	±	0	+	+	++	++	++++	++++
	Kidney	0								

of organs of mice was not disclosed since amyloidosis did not develop during the eight month period of injection of sterile sodium caseinate.

The aniline dye reactions of amyloid were constant, while the iodine reaction was variable. There was no significant variation in the intensity or the quality of the congo red or the crystal violet reactions among the several amyloid deposits. On the contrary, variations of the intensity of the iodine reaction were encountered. The variations as judged microscopically are recorded in tables 1, 2 and 8. In general, the reaction was strongly and uniformly positive only in instances of human amyloidosis. Similar strong reactions occurred in 2 instances of equine amyloidosis and 1 instance of amyloid disease in rabbits. The 2 horses in which strongly reactive amyloid was found had received the same antigen, tetanus toxin. The rabbit in which the strongly reactive amyloid was found was the only rabbit that was infected with the human tubercle

bacillus. As a rule, however, the iodine reaction was weak or negative in horses or rabbits and was not influenced by the duration of the disease, the quantity of amyloid or the solubility of the amyloid (tables 1 to 8).

TABLE 4.—*Relations Between the Quantity of Amyloid in Tissues of a Patient with Multiple Myeloma, the Intensity of the Iodine Reaction, and the Range of Solubility in Buffer Solutions*

Number	Human Myeloma Amyloid	Amount of Amyloid	Iodine Test	Observation at 24 Hours at 5 C.									
				$p_H$ 1-10		$p_H$ 11		$p_H$ 11.2		$p_H$ 11.6		$p_H$ 12	
				Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test
100	Intra-muscular	++++	++++	0	++++	0	++++	0	++	+++	0	++++	0
100	Peri-articular	++++	++++	0	++++	0	++++	0	++	+++	0	++++	0
100	Renal	+++	++++	0	++++	0	++++	0	++	+++	0	++++	0

TABLE 5.—*Relations Between the Quantity of Amyloid in Organs of Tuberculous Rabbits, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Phosphate Buffer Solutions*

No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	Solubility of Amyloid at 5 C.						
				$p_H$ 10 (24 Hr.)	$p_H$ 11 (12 Hr.)	$p_H$ 11 (24 Hr.)	$p_H$ 11.6 (12 Hr.)	$p_H$ 11.6 (24 Hr.)	$p_H$ 12 (12 Hr.)	$p_H$ 12 (24 Hr.)
1	Spleen	++++	+	0	0	0	+	++	+++	+++
	Liver	+	+	0	0	0	++	+++	++++	++++
	Kidney	+++	+	0	0	0	+	++	+++	+++
2	Spleen	++	++	0	0	0	+	++	+++	+++
	Liver	0								
	Kidney	++++	++	0	0	0	+	++	+++	+++
3	Spleen	++	++	0	0	+	++	+++	+++	++++
	Liver	0								
	Kidney	+++	++	0	0	+	++	+++	+++	++++
4	Spleen	+++	+++	0	+	+	++	++	++++	++++
	Liver	+	+++	0	0	+	++	+++	++++	++++
	Kidney	+++	+++	0	+	+	++	++	++++	++++
5	Spleen	+++	0	0	0	0	++	++	++++	++++
	Liver	++	0	0	0	0	++	++	++++	++++
	Kidney	++	0	0	0	0	++	++	++++	++++
6	Spleen	+++	0	0	++	++	+++	+++	++++	++++
	Liver	++	0	0	++	++	+++	+++	++++	++++
	Kidney	++	+++	0	++	++	+++	+++	++++	++++
7	Spleen	++++	0	0	0	0	++	++	++	++++
	Liver	++	0	0	0	0	++	++	+++	++++
	Kidney	+++	+	0	0	0	++	++	++	++++
8	Spleen	++++	++	0	0	0	++	++	+++	++++
	Liver	+++	0	0	0	0	++	++	+++	++++
	Kidney	++++	++	0	0	0	++	++	+++	+++

The several amyloids did not have the same solubility. The various solubilities are listed in tables 3 to 8. No amyloid which was soluble anywhere in the range  $p_H$  1 to 10 has yet been encountered. All amyloid matrices were soluble somewhere in the range  $p_H$  11 to 12. The amyloid in the human beings with chronic pulmonary tuberculosis was always soluble at the lower limit of this range. Usually, as shown in table 3, the

amyloid in horses was soluble at this lower limit, but in 1 instance a solution with  $p_H$  11.6, and in another instance a solution with  $p_H$  12, was necessary for dissolving the amyloid matrix. The amyloid in rabbits

TABLE 6.—*Relations Between the Quantity of Amyloid in Organs of Tuberculous Rabbits, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Alkaline Unbuffered Solutions*

Solubility of Amyloid at 5 C. in 24 Hours									
No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	N/100 NaOH	N/75 NaOH	N/50 NaOH	N/5 Ba(OH) <sub>2</sub>	Ca(OH) <sub>2</sub> (Saturated)	Str(OH) <sub>2</sub> (Saturated)
8	Spleen	++++	++	0	++	++++	0	0	+
	Liver	++	0	0	++	++++	0	0	0
	Kidney	++++	++	0	++	++++	0	0	+
4	Spleen	+++	++++	0	+++	++++	0	0	+
	Liver	+	+++	0	+++	++++	0	0	—
	Kidney	+++	+++	0	+++	++++	0	0	+
6	Spleen	+++	±	0	+++	++++	0	0	0
	Liver	++	—	0	++	++++	0	0	0
	Kidney	++	+++	0	++	++++	0	0	0
7	Spleen	++++	±	0	++	++++	0	0	+
	Liver	++	0	0	+	++++	0	0	+
	Kidney	+++	+	0	++	++++	0	0	+

TABLE 7.—*Relations Between the Amount of Amyloid in Organs of Immunized Horses, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Alkaline Unbuffered Solutions*

Solubility of Amyloid at 5 C. in 24 Hours									
No.	Equine Organ Examined	Amount of Amyloid	Iodine Test	N/100 NaOH	N/75 NaOH	N/50 NaOH	N/5 Ba(OH) <sub>2</sub>	Ca(OH) <sub>2</sub> (Saturated)	Str(OH) <sub>2</sub> (Saturated)
9	Spleen	0							
	Liver	+	±	++	++++	++++	++++	++++	++++
	Kidney	0							
10	Spleen	+	±	0	++++	++++	++++	++++	++++
	Liver	+	±	0	++++	++++	++++	++++	++++
	Kidney	0							
11	Spleen	++	++++	0	+++	++++	+	++	++
	Liver	++	++++	0	+++	++++	+	++	++
	Kidney	0							
12	Spleen	+	++	0	++++	++++	++++	++++	++++
	Liver	+	++	0	++++	++++	++++	++++	++++
	Kidney	0							
13	Spleen	++	++++	0	+++	++++	++	+	+++
	Liver	+++	++++	0	+++	++++	++	+	+++
	Kidney	0							
14	Spleen								
	Liver	+	±	0	++++	++++	++++	++++	++++
	Kidney	0							
15	Spleen	++	+	0	++++	++++	++	0	++++
	Liver	++	+	0	++++	++++	++	0	++++
	Kidney	0							
16	Spleen	+++	±	0	++++	++++	++	+	+++
	Liver	++++	±	0	++++	++++	++	+	+++
	Kidney	0							

and that in the patient with plasma cell myeloma were still more resistant to the solvent action of the buffer solutions, the matrices being never completely dissolved at 5 C. in solutions less alkaline than  $p_H$  12 (tables 4 and 5). In the correlative analysis of these variable data the conclusion



TABLE 8.—*Relations Among the Solubilities and Iodine Reactions of Amyloid Produced in Several Species by Different Apparently Causative States*

Species	Condition Apparently Related Etiologically to Amyloidosis	Organ Examined	Amount of Amyloid	pn 10		pn 11		N/5 Ba(OH) <sub>2</sub>		Saturated Ca(OH) <sub>2</sub>	
				Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test
Man	Tuberculosis.....	Liver	+++	0	++++	++++	—	0	—	0	—
Man	Tuberculosis.....	Liver	+++	0	++++	++++	—	+	—	0	—
Man	Tuberculosis.....	Liver	++	0	++++	++++	—	0	+	0	+
Man	Myeloma.....	Muscle	+++	0	++++	0	++++	0	+	0	+
Horse	Tetanus toxin.....	Spleen	++	0	++++	++	+	+	+	++	—
Horse	Tetanus toxin.....	Liver	++	0	++++	++	+	+	+	++	—
Horse	Tetanus toxin.....	Spleen	++	0	++++	++++	—	++	—	+	—
Horse	Tetanus toxin.....	Liver	+++	0	++++	++++	—	++	—	+	—
Horse	Several toxins.....	Spleen	+	0	++	++++	—	++++	—	++++	—
Horse	Several toxins.....	Liver	+	0	++	++++	—	++++	—	++++	—
Horse	Diphtheria and tetanus toxins.....	Spleen	++	0	+++	+++	+	+++	—	+	—
Horse	Diphtheria and tetanus toxins.....	Liver	+++	0	++	+++	+	++	—	+	—
Rabbit	Tuberculosis.....	Spleen	+++	0	++++	+	+	0	++	0	+
Rabbit	Tuberculosis.....	Liver	+	0	+++	+	—	0	+	0	+
Rabbit	Tuberculosis.....	Kidney	+++	0	+++	+	—	0	+	0	+
Rabbit	Tuberculosis.....	Spleen	++	0	+++	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Kidney	+++	0	+++	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Spleen	++	0	+++	++	+	0	+	0	—
Rabbit	Tuberculosis.....	Liver	+	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Kidney	+++	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Spleen	++	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Liver	+	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Kidney	+++	0	++	0	—	0	—	0	—
Rabbit	Tuberculosis.....	Kidney	+++	0	++	0	—	0	—	0	—

was drawn that constant solubility was the rule when the variables of species and apparent cause were fixed. There was no correlation between the solubility of amyloid and the duration of the amyloidosis, the quantity of amyloid or the intensity of the iodine reaction.

The differences in solubility of amyloid, as disclosed by use of buffered solutions, were again illustrated by use of alkaline unbuffered solutions as solvents (tables 6, 7 and 8). In aqueous sodium hydroxide solution every amyloid was soluble at some concentration in the range hundredth to fiftieth normal. In general, the types of amyloid which dissolved at  $p_H$  11 were soluble at the lower alkaline limit of this range, and those which dissolved at  $p_H$  12 were soluble only at the upper alkaline limit of the range. Other alkaline solutions had variable actions. As a rule, strontium hydroxide was more effective as a solvent than barium hydroxide, and calcium hydroxide was the least effective. These reagents did not dissolve the amyloid of tuberculous rabbits or, as shown in other studies, the amyloid occurring in human beings with tuberculosis (tables 6 and 8). They proved to be moderately efficient as solvents for the amyloid in immunized horses, especially when the quantity of amyloid in the tissues was small (table 7).

Irrespective of the nature of the solvent, the affinities of the amyloid matrix for crystal violet and congo red persisted until the last trace of amyloid was dissolved. On the contrary, the iodine reaction which initially was positive often became negative in the presence of various reagents, even though the amyloid matrix otherwise exhibited no significant change (tables 4 and 8). In all instances the intensity of the iodine reaction at neutrality was fully maintained throughout the range  $p_H$  7 to 10. Positive iodine reactions usually became negative during extraction of the tissue with aqueous fifth-normal barium hydroxide solution, a saturated solution of calcium hydroxide or a phosphate buffer solution of  $p_H$  11. The loss of the capacity of the amyloid matrix to combine with iodine to give a positive reaction after treatment with these reagents was not specifically related to the solubility of the entire matrix or to any other factor which could be discovered.

#### COMMENT

These studies show some relationships between abnormal conditions which served as apparent causes of amyloid disease, the species in which the disease occurred, the solubilities of the matrix and the staining qualities of the matrix.

Chronic destruction of tissue with repair was common to all apparent etiologic states. This characteristic was especially prominent in most rabbits with experimental tuberculous infections, in human beings with pulmonary tuberculosis and in the patient with plasma cell myeloma. In other instances, especially those in which amyloidosis developed in

horses during courses of active immunization, the degree of tissue injury was minimal and was localized to parenteral sites of injections of antigens. If tissue injury with repair had anything to do with the development of amyloid disease, it is certain that the degree of injury was not of first importance. Likewise, the duration of the tissue injury beyond a variable minimum limit of time had no consistent quantitative bearing on the development and progress of amyloidosis. This variable interval of time between the onset of the abnormal state and the first appearance of amyloid in the tissues depended on at least two factors. One factor was inherent in the species, for the interval of time was shortest with rabbits, of medium length with horses and of greatest length with human beings. A second factor was the nature of the abnormal condition which served as an apparent cause of amyloidosis. For example, rabbits with tuberculosis usually acquired amyloid disease within a period of seven to twelve months. In the experiment in which the tuberculous rabbits were given subcutaneous injections of tuberculin, amyloid disease was severe at the end of three months. In the control rabbits, which had a similar amount of tuberculosis and which received no tuberculin, amyloidosis did not develop during the three month period of infection. Thus, the usual course of development of amyloid disease in tuberculous rabbits was shortened and the quantity of amyloid deposited was increased by subcutaneous injections of tuberculin. This effect of tuberculin in rabbits is in accord with the well known observation that in horses repeated injections of certain antigens lead to the development of amyloid disease in the absence of infections or significant injury of tissue.<sup>4</sup>

It seems, therefore, that persistent or repeated stimulation of immune mechanisms is a fundamental factor in the genesis of amyloid disease. This factor was common to all except 1 of the instances of amyloid disease which we have studied. The exception was the case of plasma cell myeloma, and other exceptions have been described in the literature. In these instances there has been no proof that immune mechanisms had not been actively stimulated, and until such proof is offered they cannot logically be regarded as true exceptions to the rule.

A more positive argument against the fundamental importance of antigenic stimulation in the genesis of amyloid disease is the frequent absence of the disease in the presence of intensive prolonged stimulation of immune mechanisms. This raises the question of the qualitative and quantitative aspects of immunity. So far as qualitative aspects are concerned, the present studies illustrate conspicuous differences in effectiveness among antigens. In horses, tetanus toxin was most effective in the production of amyloid disease, while killed pneumococci were not effective. The ineffectiveness of killed pneumococci was again illustrated by experiences with rabbits, all of which received many injections over a

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4. Arndt, H. J.: *Verhandl. d. deutsch. path. Gesellsch.* **26**:243, 1931.

period of four months without the development of amyloidosis.<sup>5</sup> Likewise, mice which were given injections of a solution of sterile sodium caseinate did not acquire the disease. Despite the fault of comparing the effects of different antigens in species of different susceptibilities, the conclusion may be justified that in a suitably controlled experiment antigens with different qualities would have different capacities for producing conditions leading to amyloid disease. From the quantitative aspect of immunity the more important theoretic considerations would be the level of the serum antibody titer, the quantity of circulating antigen, the insolubility of the antigen-antibody precipitate and the length of the period of immunization.<sup>6</sup> It remains for experiments to show whether or not there are precise correlations between these factors and the occurrence of the disease.

The implied order of diminishing species susceptibility to the disease, namely, rabbit, horse, man and mouse, may be more apparent than real since the conditions were not the same among the several species. If this is a true order of susceptibility, the experimental data offered no explanation.

The order of susceptibility of organs among the several species may be regarded as a more reliable result of this study. Except for the instance of atypical amyloidosis complicating plasma cell myeloma, the order was fairly constant in each species and was independent of variables such as apparent causative condition and duration of the amyloidosis. The interesting studies of Dick and Leiter<sup>7</sup> indicate that there are exceptions to this general conclusion and that resorption of amyloid is an important factor in the regulation of the observed distribution in organs. The most conspicuous examples of susceptibility of organs were the massive deposits of amyloid in the spleens of many tuberculous rabbits and the absence of amyloid deposits in the renal glomeruli of horses. The latter observation was surprising because in man and especially in rabbits glomerular arterioles are elective sites for the deposition of amyloid. It is possible that the species variation in arteriolar dimensions has something to do with the observed differences.

The variations in the solubility of amyloids were independent of any apparent factor. As a rule, with a given abnormal state in a given species the solubility was constant and was independent of the duration of the abnormal state, the quantity of amyloid or the intensity of the iodine reaction. But with different abnormal states in a given species, as illustrated by the equine and the human material, the amyloid deposits

5. Repeated injections of living pneumococci of type III, however, will lead to the onset of amyloid disease in rabbits within a period of eight months (Angevine, D. M., and Freund, J.: Personal communication to the authors).

6. Letterer, E.: *Virchows Arch. f. path. Anat.* **293**:34, 1934. Eklund, C. M., and Reimann, H. A.: *Arch. Path.* **21**:1, 1936.

7. Dick, G. F., and Leiter, L.: *Am. J. Path.* **17**:741, 1941.

had different solubilities. Though these differences were not conspicuous, they were detectable and offer some support to the thesis that amyloid is a product of variable composition.

The concept that amyloid may have a variable composition is in agreement with the differences in the intensity of the iodine reaction. The differences which were encountered in the present study could not be correlated satisfactorily with any special factor. Only human amyloid consistently gave a strong positive reaction. In the amyloid of rabbits and horses the reaction was usually weakly positive or negative. If these variations in the reaction are eventually to be explained by the presence of some compound in the amyloid matrix, it may be inferred from the data that the reactivity of the compound can be modified without producing any detectable quantitative change in the matrix or qualitative change in the affinities of the matrix for crystal violet and congo red. In view of the experimental modifications of the quality of the reaction *in vitro*, doubt remains as to whether the induced changes in the reaction were due to a change in some compound which remained in the matrix or to removal of some minor component by solvents which did not dissolve the principal part of the matrix. If some compound in the matrix was changed, the change was irreversible. If some minor component was removed from the matrix, it is possible that the component was the sulfate-bearing polysaccharide recently isolated from amyloid. Though possible, it is unlikely that extraction of the polysaccharide was responsible for the loss of the iodine reaction, since the polysaccharide is not extractable under some conditions which lead to a change of the iodine reaction. Though this problem has not been solved, a first step in the solution has been made by specification of methods by which the iodine reaction and the principal part of the matrix may be modified in a differential way with several alkaline reagents. If possible, at least for chemical purposes, complementary methods for the differential modification of the matrix and the iodine reaction with acid reagents should be made available.

Though all types of amyloid disease were not studied, the data define several simple properties of amyloid in special cases. The definition of these properties may facilitate a more complete analysis of the composition of amyloid in man and two species of experimental animals. Furthermore, the data offer standards by which the different amyloids may be crudely distinguished from one another and from morphologically similar hyaline intercellular substances. Among these similar substances the most common are those which occur in the vascular disease known as arteriosclerosis. As these have not been defined by other than morphologic methods, it is possible that some of the confusion which centers about differences between hyaline and amyloid "degeneration" can be dispelled by appropriate studies of the solubilities of the products in

question.<sup>8</sup> This would seem to be the simplest and most direct approach to the objective of segregation of the several products in purified form and of final analysis of their genesis.

#### SUMMARY

A comparative study of amyloid disease in man, horses and rabbits was made. In man the apparent cause of the disease was chronic pulmonary tuberculosis or plasma cell myeloma; in horses, it was prolonged immunization with one or more noninfectious antigens, and in rabbits, chronic tuberculosis, which in several animals was complicated by injections of heat-killed tubercle bacilli or old tuberculin. Amyloid disease did not develop in horses and rabbits during prolonged immunization with heat-killed pneumococci, nor could the disease be produced in mice by repeated injections of sterile sodium caseinate.

Amyloid disease developed more readily in rabbits than in horses or in man. The most effective method of producing the disease in rabbits was that of injecting tuberculin into animals that had active bovine tuberculosis. The most effective method of producing it in horses was that of injecting tetanus toxin. In a given species the quantity of amyloid depended primarily on the nature of the apparent causative condition and secondarily on the duration of this condition.

There was a species variation in the quantitative partition of amyloid in the spleen, the liver and the kidney. Splenic amyloid was most voluminous in rabbits. Hepatic amyloid was most prominent in man. Renal amyloid was more abundant in rabbits than in man and was never found in horses.

The solubility of different amyloid matrices varied. All were soluble somewhere in the range  $p_H$  11 to 12. Various ones had different solubilities in solutions of barium, calcium and strontium hydroxide. The solubilities were constant in a given species in the presence of a given apparently causative condition, and they did not vary with the age of the amyloid deposits, the quantity of amyloid or the intensity of the iodine reaction.

All amyloid deposits gave similar aniline dye reactions, but the intensity of the iodine reaction varied. Human amyloid always gave a near maximum positive iodine reaction, while the reaction of amyloid in horses and rabbits was usually weak or negative. The intensity of a positive reaction was not diminished during extraction of amyloid at  $p_H$  10, but positive reactions became negative at  $p_H$  11 or during extraction with barium, calcium or strontium hydroxide. The change from positive to negative reactions often occurred, especially during extraction with calcium hydroxide, without any appreciable change in the quantity of amyloid or the affinities of the matrix for crystal violet and congo red.

8. Leupold, E.: *Ergebn. d. allg. Path. u. path. Anat.* (pt. 1) **21**:120, 1925.

# THE VENOUS VALVES IN THROMBOANGIITIS OBLITERANS

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It is well established that thromboangiitis obliterans attacks the veins of the extremities as extensively as it does the arteries. It would be indeed surprising if the delicate valves of the veins should escape involvement of some kind. Yet in the extensive literature on thromboangiitis obliterans we have found only a parenthetical mention of valvular changes.<sup>1</sup>

In previous publications we have demonstrated that in ordinary thrombophlebitis<sup>2</sup> and in varicose veins<sup>3</sup> there is extensive involvement of the valves, which may account for much of the disturbance of the venous return. We may similarly infer that if damage of the valves were demonstrated in thromboangiitis obliterans it would help to explain some of the circulatory difficulties in this disease.

Accordingly, we have studied the blood vessels of lower extremities from a series of patients with thromboangiitis obliterans and have observed that the venous valves are seriously damaged.

In order that the cases included here may be accepted as authentic instances of thromboangiitis obliterans, we shall indicate what criteria we have used in making the diagnosis. The absolute microscopic criterion of thromboangiitis obliterans is the presence of an intraluminal granuloma consisting of fibroblasts, lymphocytes, macrophages, and multinucleated giant cells resembling the foreign body type (fig. 1). This lesion may most often be found localized in segments of the superficial veins. It is met with uncommonly in the deep veins and rarely in the arteries. But the vessels lacking this pathognomonic tissue often show fibromuscular intimal thickening or thrombosis, while some segments show no evident abnormality. The diagnosis is strongly indicated when there is thrombosis of both arteries and veins with fibrosis of the

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1. von Winiwarter, F.: *Arch. f. klin. Chir.* **23**:202, 1879. Popoff, N. W.: *Arch. Path.* **18**:295, 1934.

2. Edwards, E. A., and Edwards, J. E.: *Surg., Gynec. & Obst.* **65**:310, 1937.

3. Edwards, J. F., and Edwards, E. A.: *Am. Heart J.* **19**:338, 1940.

perivascular tissue, often including the accompanying nerves. Infiltration of macrophages in all layers of the vein, together with reduplication of the elastic laminas, and scarring, with interruption of the mural structure, are also very suggestive.

In the absence of the specific granulomatous lesions, the clinical status is a great aid in establishing the diagnosis. Classically, one finds evidence of arterial insufficiency in a man under 40 who is a heavy smoker of tobacco, and who shows evidence of migrating phlebitis or gives a history of this condition. In the absence of the specific changes,

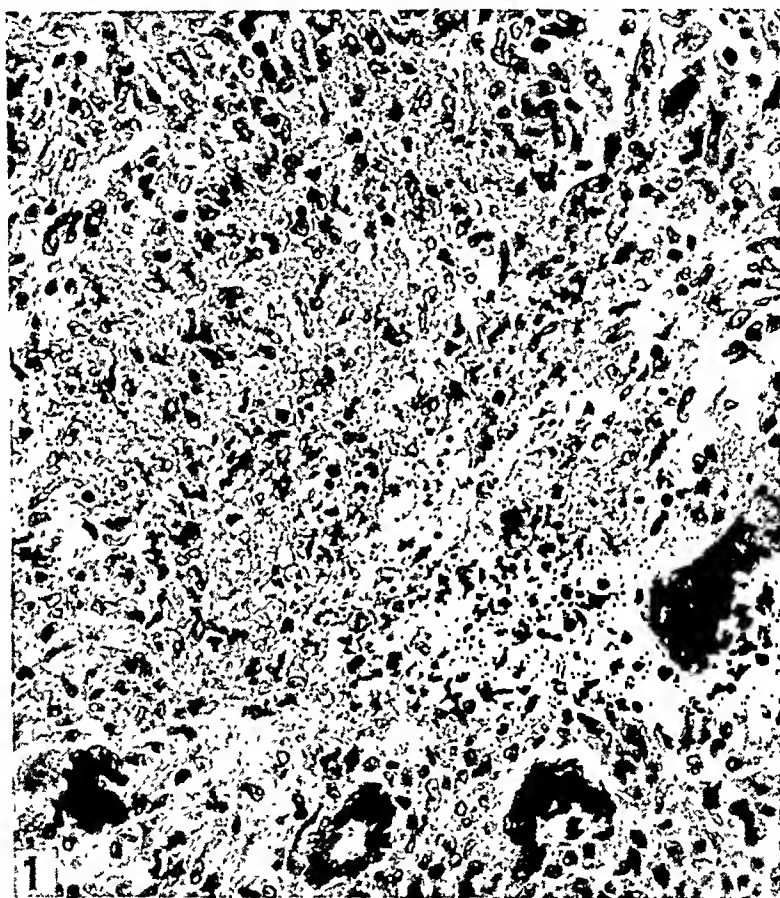


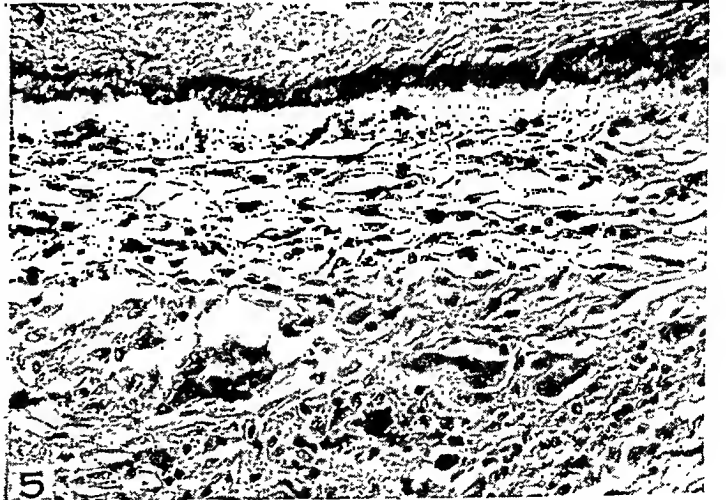
Fig. 1.—The intraluminal granuloma of thromboangiitis obliterans. Besides the multinucleated giant cells, there are numerous macrophages, fibroblasts and lymphocytes. A central area of necrosis is present.  $\times 310$ .

the association of such circumstances with the aforementioned suggestive pathologic changes makes almost certain the diagnosis of thromboangiitis obliterans.

The blood vessels used in this study were obtained from 23 amputated extremities and 3 bodies on which autopsies had been done.<sup>4</sup> The

4. Dr. Tracy B. Mallory gave us the opportunity to include in our study the material from the department of pathology of the Massachusetts General Hospital, and Dr. Richard Wadsworth the material from a patient at the Metropolitan State Hospital, Waltham, Mass.





Figures 2 to 5

(See legends on opposite page)

photomicrographs shown here were made from the vessels obtained in 9 cases. In 5 instances the specific lesion was present; in the following paragraphs will be found the significant clinical features in the remaining 4 cases.

CASE 1.—A man admitted to the Boston City Hospital stated that the onset of pain in the extremities occurred when he was 30 years old. He smoked cigarettes, but the number consumed is not recorded. In the twelve years between the first symptoms and his appearance in the hospital he had submitted to amputation successively of the left great toe, the remaining toes, part of the left foot, the lower third of the leg, the middle third of the leg, the lower part of the left thigh and finally the middle of the left thigh.

During this time he had begun to have pain in the right foot. Examination showed no pulsation below the level of the femoral artery. The urine showed no albumin or sugar; the Hinton test of the blood was negative. A series of sensory nerve crushings had been done with some relief, and this was followed by lumbar sympathectomy on the right side. He improved and was promptly discharged. He returned nine months later because of an ulcer of the foot. This progressed, and the right leg was amputated at the junction of its middle and upper thirds. The tissue examined in this study was obtained from this limb.

CASE 2.—A man entered the Boston City Hospital at 34 years of age. He smoked cigarettes, but the number consumed is not recorded. He had had pain and claudication for six months. There were no pedal pulses, but the popliteal artery pulsated. Cyanosis was marked. In the course of five days his condition progressed to frank gangrene and mid thigh amputation was done on the left extremity. Examination of the vessels showed old thrombosis of the posterior tibial artery with recent thrombosis of the popliteal artery.

He returned four months later because of thrombophlebitis of the right saphenous vein. The foot was pale and perspiring. While under observation the femoral vein was likewise involved in thrombophlebitis. A right lumbar sympathectomy was done, and the patient was discharged improved. He came back ten months after his second admission because of pain in the right great toe. The toe was swollen, purple and cold. There were no pulses below the femoral level. The Hinton test of the blood was "doubtful." The urine showed no sugar. He failed to improve after conservative treatment, and a mid thigh amputation was done. Old and recent thrombosis of the posterior tibial vessels was found. The vessels of this limb were included in the present study.

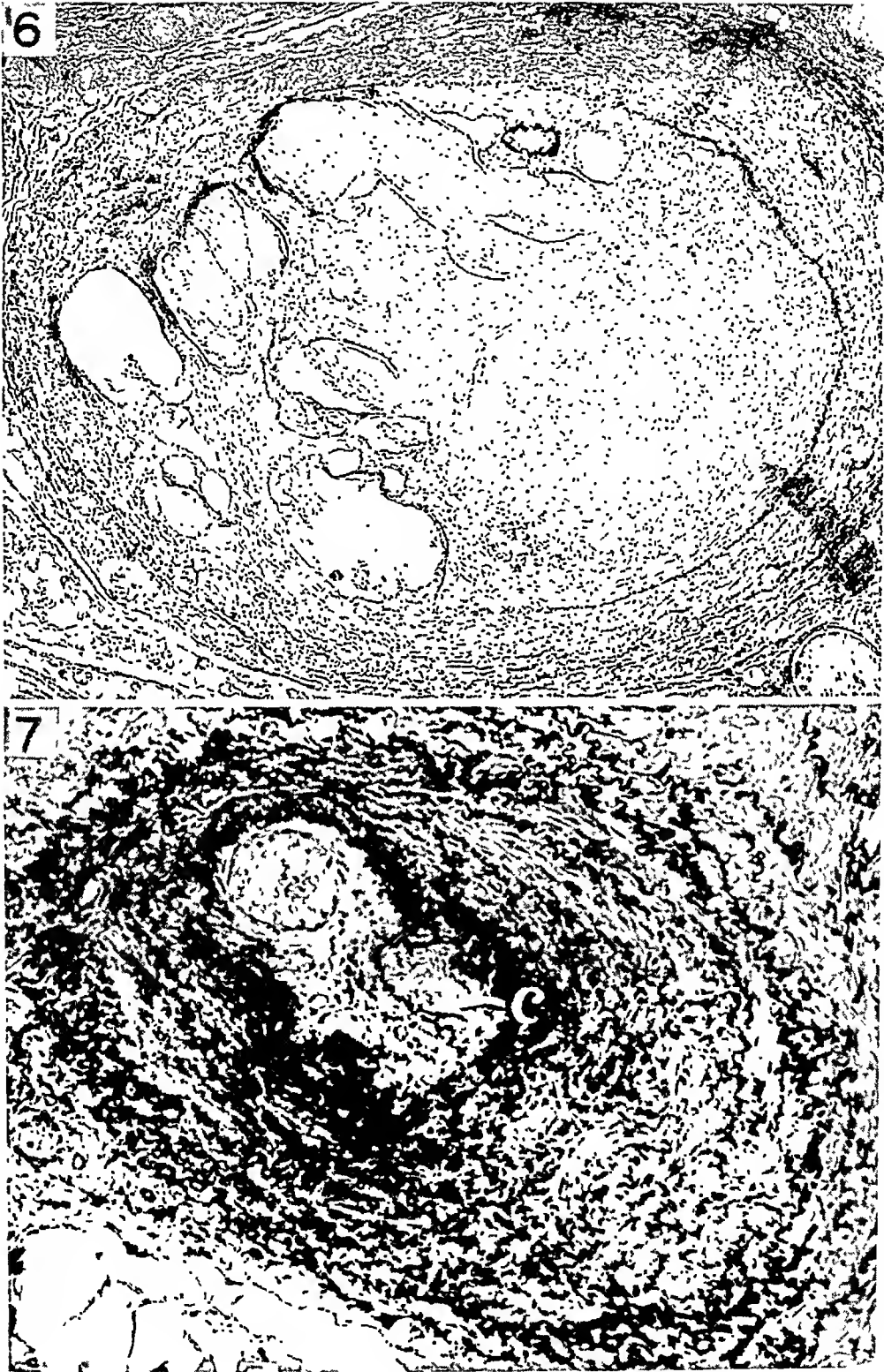
#### EXPLANATION OF FIGURES 2 TO 5

Fig. 2.—The involvement of a valve in the granulomatous inflammation. Almost an entire cusp is incorporated in the tissue which fills the valve sinus. Phosphotungstic acid-hematoxylin stain.  $\times 124$ .

Fig. 3.—The interadhesion of the cusps brought about by fibrous tissue. It represents the end stage of either the inflammatory process or the local thrombosis. The typical granuloma was present in other sections of this case.  $\times 112$ .

Fig. 4.—Early incorporation of the valve cusps in a thrombus that is being recanalized, engrafted on an inflammation.  $\times 37$ .

Fig. 5.—Portion of a cusp from the previous figure, showing its relation to the granuloma and the thrombus.  $\times 233$ .



Figures 6 and 7  
(See legends on opposite page)

CASE 3.—A man was first seen at the Massachusetts General Hospital at the age of 37. He had had migrating phlebitis of the left foot for four months. There were no pedal pulses on this side. The toes were cyanotic and cold. No calcification of the arteries was seen in the roentgenograms. The Wassermann test of the blood was negative, and the urine showed no sugar. He admitted smoking since the age of 12; at first he used 10 to 15 cigarets a day, but admitted smoking as much as 6 pipefuls of tobacco plus 20 cigarets a day at the time of admission. He was seen at short intervals at the hospital for ulceration of the toes of both feet, and had an amputation of the right great toe two years after his first admission, which was followed in the next year by gangrene of the foot necessitating a Gritti-Stokes amputation of the right leg.

One year later, when he was 41, this same procedure was carried out on the left side. Vessels from the amputated limb were used in this study. The disease progressed to ulceration and development of gangrene in the fingers of both hands. The radial arteries at first showed diminution in size and at his last admission, at the age of 46, he had lost several fingers and parts of the remaining digits. There was no pulsation in the vessels at the wrists or the elbows.

CASE 4.—A man was first seen in the Massachusetts General Hospital at the age of 24 for pain and swelling of the right foot. He had had claudication in the right calf for one year, and four weeks before admission he had symptoms suggesting arterial thrombosis of the leg; he presented gangrene of the toes. The Wassermann test of the blood was negative; the urine showed no sugar. The vessels did not appear calcified in the roentgenogram. He admitted smoking tobacco since the age of 12; he had smoked 20 cigarets a day for ten years but had increased this to 30 to 40 cigarets a day for the two years preceding admission.

A Gritti-Stokes amputation of the right leg was done; thrombi of varying ages were found in the arteries and veins of this limb. He suffered migrating phlebitis of the left thigh and the right stump during the convalescence. Two years later a guillotine amputation of the left leg was necessary because of gangrene of the toes and infection. Vessels from the amputated part were used in the present study. Reamputation at the level of the lower part of the thigh was later necessary. This was followed by intractable pain in the stump, which was finally treated by an intrathecal injection of alcohol. The amputation stump was amputated in turn because of slough. Later that year the patient had a cerebrovascular accident with paralysis of the right side of the face and the right upper limb. It was learned that he died suddenly the following year at the age of 28; his physician believed the death was caused by pulmonary embolism.

#### EXPLANATION OF FIGURES 6 AND 7, SHOWING THROMBOTIC CHANGES IN THE VENOUS VALVES

Fig. 6 (case 2).—Organization and early recanalization of a thrombus in a vein have almost completely destroyed one cusp and have seriously disrupted the second. The typical granuloma was not found in this case.  $\times 38$ .

Fig. 7.—The elastica of a cusp, C, lies within an organized thrombus. The enormous proliferation of the elastica of the vessel wall is often characteristic of thromboangiitis obliterans. The typical granuloma was present in other sections.  $\times 150$ .

## THE VALVE CHANGES

The disease process involving the valves is found to be a part of that involving the blood vessels in general. In order, therefore, to elucidate the lesions of the valves, we shall have to discuss to some extent the general pathologic aspects of this disease.

Three types of lesions are found in the blood vessels and valves: (1) inflammation, (2) thrombosis and (3) dilatation. In any one patient, and indeed in any one section of a vascular bundle, all three changes may exist side by side in the different veins and arteries.<sup>5</sup>

*Inflammation.*—The inflammation in thromboangiitis obliterans is characterized by an involvement of the entire vessel wall and perivascular region. The exudate contains many macrophages and lymphocytes and ordinarily but few polymorphonuclear leukocytes. The intima is thickened by hypertrophy of the elastica interna and by the growth of muscle cells and, particularly in the veins, by a focal inflammation with projection into the lumen of a nodular collection of inflammatory exudate. As already mentioned, this intraluminal tissue may or may not be a typical granuloma containing multinucleated giant cells. Buerger<sup>6</sup> originally described this tissue as developing within an organizing thrombus, but it probably precedes the thrombosis, since it can be seen in the absence of any thrombus.

Buerger indeed held that the organization of thrombi, which are widespread in this disease, accounts for the thickening of the intima and the intraluminal projection of tissue of whatever variety. One must, of course, admit that particularly in the veins there may be areas where the intima is thickened as the result of the recanalization of an obstructive thrombus or the simple organization of a parietal thrombus. But we cannot agree with Buerger that the greater part of this tissue is of such origin. There is ample evidence, too extensive to be reviewed here, that a thickening of vascular intima by proliferation of its cells or by inflammatory exudation occurs as a reaction to a great variety of stimuli. On the other hand, it is difficult to conceive of such universal thrombosis as would be necessary to account for the initial thickening wherever it is encountered. One may presume that under such conditions the clinical symptoms would be infinitely more severe than those one now sees in the disease. Finally, this tissue differs histologically from that which arises by the organization of a thrombus. Blood pigment is not seen; the tissue is not vascular, and neither is there any great increase in vascularity of the vessel wall, which, contrariwise, is so striking in the presence of a visible thrombus in this disease.

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5. Except when noted, all the preparations shown were made with Verhoeff's elastic tissue stain overlaid by Van Gieson's stain. Mallory's aniline blue and phosphotungstic acid-hematoxylin stains were used in some cases.

6. Buerger, L.: *The Circulatory Disturbances of the Extremities*, Philadelphia, W. B. Saunders Company, 1924.

The valve cusp alone may be the site of inflammation with resultant thickening and with disorganization of its structure. More commonly, the cusp is free of primary changes but becomes involved in the inflammation of the adjacent venous wall (fig. 2). In this way the cusp may be anchored in the exudate, becoming grossly inefficient or completely useless.

Resolution of the inflammation probably never allows the valve to return to normal, even if the cusp has suffered no intrinsic damage. Varying degrees of fibrosis result, leading to adhesion of the cusps to the intima of the venous wall or to each other, and thus their normal excursion is interfered with (fig. 3).

Moreover, the inflammation is often associated with thrombosis of the involved segment (figs. 4 and 5), which, as will be seen in the following section brings its own serious damage to the valve.

*Thrombosis.*—Thrombosis is a second prominent finding in thromboangiitis obliterans. The organization of the thrombus is accompanied by an unusually prominent vascularization of the vessel wall. Yet the internal elastic lamina remains prominent, though perforated by the vessels passing from the vasa vasorum to the thrombus. Recanalization occurs as in thrombi of any origin.

These processes of organization and recanalization affect the valves in the same deleterious manner as we have demonstrated them to be affected in thrombosis from other causes. The cusp, lying within the thrombus, shows early disruption of its structure by virtue of its participation in the organization of the thrombus. The capillaries at its base proliferate and extend toward its free margin; its endothelium disappears, and fibroblasts course through its substance. As collagen is laid down in the organizing thrombus, the cusp is incorporated in the new tissue. From this time on, its identity can usually be distinguished only with the aid of stains for elastic tissue, which demonstrate its elastic layer. But the elastica itself early shows twisting, reduplication, and interruption by the invasion of capillaries continuous with those of the adjacent organizing thrombus.

As recanalization proceeds, the ever widening blood channels progressively break down the structure of the cusp. When recanalization is complete, the only recognizable elements of the cusp are bits of its elastic tissue incorporated in the walls of the newly formed channel or channels (figs. 4, 5, 6 and 7).

In places, a thrombus may be deposited along the venous wall, without completely filling the lumen. Such mural thrombi are apt to be formed in the valve sinuses, i. e., to the outer side of the cusps. Here again the cusp may become adherent to, or incorporated within, the organizing thrombus, to an extent depending on the degree of its contact with the thrombus (fig. 8).

*Dilatation.*—Many valves show changes identical with those we have demonstrated in the varicose saphenous vein. In illustrating this lesion in thromboangiitis obliterans, we have chosen examples only in the deep veins, avoiding the superficial veins which might well have been subject to spontaneous varicose change.

A vein tends to be elliptic in cross section, with the cusps of the valve stretched across the long axis.<sup>7</sup> The dilatation at first takes place in this axis and acts on the valve commissure, which is the site of attachment of the two cusps to the wall of the vein. Early there is a thinning

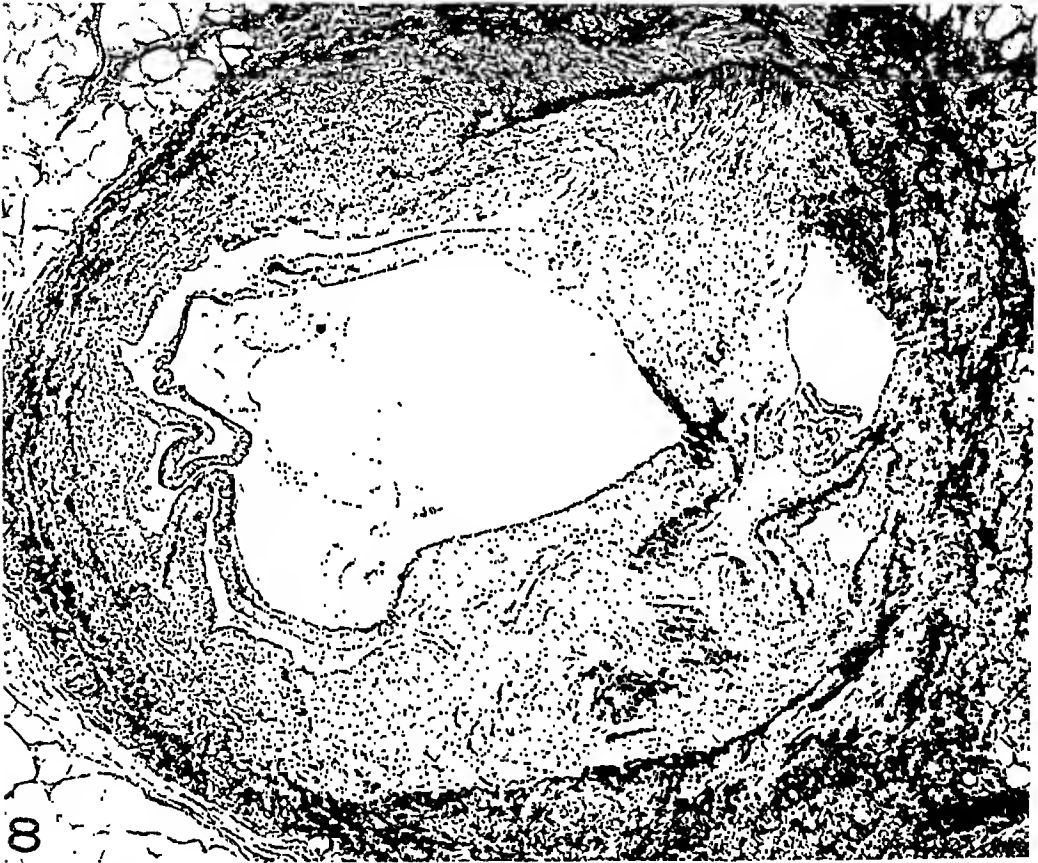
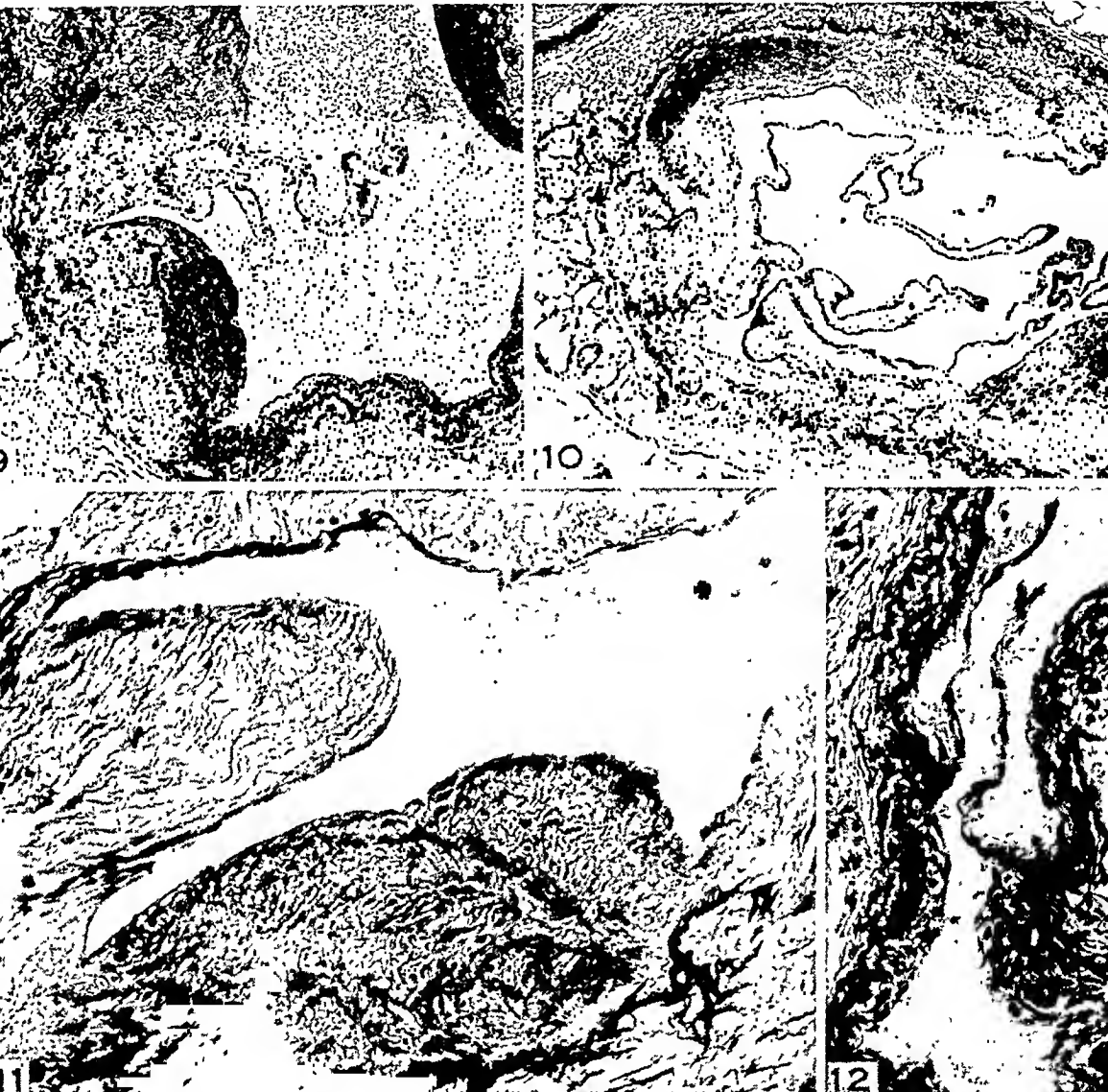


Fig. 8 (case 4).—Extensive adhesions of the valve cusps to an organized mural thrombus, with destruction of most of the valve. The typical granuloma was not found in this case.  $\times 50$ .

of the mound of tissue normally present here, with the gradual development of a lateral cleft between the attachments of the cusps (fig. 9). As the dilatation proceeds, it may deepen the cleft into an evagination (fig. 10), and at about this time the vein widens in its shorter diameter. This increases the distance between the adjacent cusps. Both the evagination and the separation of the cusps thus result in incompetence of the valve; the first by producing a valveless channel lateral to the cusps, the second by preventing their approximation.

7. Edwards, E. A.: Anat. Rec. 64:369, 1936.





EXPLANATION OF FIGURES 9 TO 12, SHOWING DILATATION CHANGES  
IN THE VENOUS VALVE

Fig. 9 (case 1).—The commissure of the valve shows disappearance of its usual mound of tissue. A niche has formed in its place, between the cusp attachments. The typical granuloma was not found in this case.  $\times 41$ .

Fig. 10 (case 3).—Continued dilatation has widened the defect between the cusp attachments. The elastica connecting the cusps is thinned out. The typical granuloma was not found in this case.  $\times 25$ .

Fig. 11.—The region of the commissure after extreme separation of the cusps. A mass of fibroblasts and muscle cells has grown into the defect. From the same patient as figure 3.  $\times 235$ .

Fig. 12.—Longitudinal section of a valve, showing fibroblasts and muscle cells extending onto the contact surface of each cusp. From the same patient as figure 9.  $\times 245$ .



Frequently these primary changes are associated with a reparative process which tends, though in vain, to fill in the anatomic gap caused by the processes of evagination and dilatation. This consists of a deposit in the defect of a mass of tissue composed of fibroblasts, large numbers of smooth muscle cells, elastic tissue and collagen (fig. 11). The tissue often grows along the contact surfaces of the cusps, resulting in further thickening and rigidity of the valve (fig. 12). Capillaries and blood pigment are characteristically absent. This, together with the presence of considerable smooth muscle, speaks very strongly against this tissue's having its origin in an organized thrombus.

We judge that the dilatation evident at the valve site is secondary to obstruction at proximal levels. This brings to mind the work of Thoma, who obtained intimal proliferation *on both sides* of experimental ligatures of both veins and arteries.<sup>8</sup> It was his thesis that slowing of the blood stream operated to produce this change whether or not dilatation was present. Areas of obstruction are of course widely distributed in the veins and arteries of the limbs in thromboangiitis obliterans. We may well inquire whether here as well the obstruction may not be responsible for much of the intimal proliferation found in the disease, of which the described reaction changes at the valve sites are but a part. We hope to inquire further into this matter.

#### SUMMARY

Examination of the blood vessels of the lower extremities shows that thromboangiitis obliterans damages the venous valves extensively and seriously. The lesions involving the valves are part of the changes in the blood vessels in general—first, inflammation; second, thrombosis, and third, dilatation secondary to the obstruction by the inflammation or thrombosis.

The valves may be disrupted by the inflammation. Their excursion may be limited by their involvement in the inflammatory exudate in the valve or in the vessel wall, or by the formation of adhesions.

In obstructive thrombosis the valve is destroyed by the organization and recanalization, any remaining portions being incorporated in the walls of the channel or channels. In mural thrombosis the cusps may be incorporated in the organizing tissue or their excursion limited by thickening and adhesion.

The dilatation of the veins distal to areas of obstruction is associated with relative incompetence of the valve. The growth of reparative tissue often additionally thickens the cusps and makes them rigid. The thesis is presented that this may be but one part of a widespread proliferative change in thromboangiitis obliterans secondary to the obstruction of the blood vessels.

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8. Thoma, R.: Virchows Arch. f. path. Anat. **204**:1, 1911.

# CHEMOTHERAPY OF EXPERIMENTAL STREPTOCOCCIC PERICARDITIS

A COMPARISON OF SULFANILAMIDE AND AN ACETYLATED  
DERIVATIVE OF 4,4'-DIAMINODIPHENYLSULFONE HYDRO-  
CHLORIDE IN THE TREATMENT OF EXPERIMENTAL  
BETA HEMOLYTIC STREPTOCOCCUS PERI-  
CARDITIS IN THE RABBIT

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Even though the cure of certain bacterial infections in man by members of the sulfonamide group of compounds represents a great advance in chemotherapy, the efficacy of these drugs is by no means ideal. Buttle,<sup>1</sup> Fourneau<sup>2</sup> and their co-workers reported a considerably higher degree of chemotherapeutic activity for 4,4'-diaminodiphenylsulfone than for sulfanilamide in beta hemolytic streptococcus infections of mice. These reports were confirmed by other groups of investigators. Thus, Raiziss and his collaborators<sup>3</sup> have shown that this compound is ten times as effective as sulfanilamide in mice infected intraperitoneally with the C203 strain of the beta hemolytic streptococcus. The corresponding diacetyl derivative of this sulfone compound, although less toxic, is also less active. From these and other reports,<sup>4</sup> as well as from a thorough exploration of the literature in general, it appears that 4,4'-diaminodiphenylsulfone is probably the most therapeutically effective compound against the hemolytic streptococcus yet to be synthesized.

Unfortunately, the toxicity of this compound is so great as to forbid its use in both experimental and human streptococcic infections. Thus, in our preliminary trial experiments single daily doses of 0.3 Gm. of 4,4'-diaminodiphenylsulfone were administered by mouth for three to

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From the Eugene Littauer Memorial and Fulton County Laboratories of the Nathan Littauer Hospital.

1. Buttle, G. A. H.; Stephenson, D.; Smith, S.; Dewing, T., and Foster, G. E.: *Lancet* **1**:1331, 1937.

2. Fourneau, E.; Tréfouël, J.; Nitti, F., and Bovet, D.: *Compt. rend. Acad. d. sc.* **204**:1763, 1937.

3. Raiziss, G. W.; Severac, M.; Moetsch, J. C., and Clemence, L. W.: *Proc. Soc. Exper. Biol. & Med.* **39**:339, 1938.

4. Bauer, H. S., and Rosenthal, S. M.: *Pub. Health Rep.* **53**:40, 1938.  
Feinstone, W. H.; Bliss, E. A.; Ott, E., and Long, P. H.: *Bull. Johns Hopkins Hosp.* **62**:565, 1938.

four days, starting twenty-four hours after the production of beta hemolytic streptococcus pericarditis in rabbits. Death was delayed only three to four days beyond the control period. Repeated attempts to increase the dose of the drug in the infected animals led to such severe acute toxic reactions that they were quickly abandoned.

Relatively few attempts<sup>5</sup> have been made to reduce the toxicity of this drug without excessive loss of activity by modification of its active amino groups through acetylation and through use of acyl substituents and Schiff bases. Although the derived products are less toxic, they are not as effective as the parent compound. Recently Roblin and co-workers<sup>6</sup> synthesized a new series of derivatives of 4,4'-diaminodiphenylsulfone, many of which are closely related to the original compound and two of which exhibited a high antistreptococcic action in mice.

In consideration of the high degree of antistreptococcic activity of 4,4'-diaminodiphenylsulfone, it was deemed desirable to diminish its toxicity without excessive reduction of its effectiveness. By preliminary treatment with 1 molar hydrochloric acid in vitro, it was converted into a hydrochloride with an increase in aqueous solubility; thus intestinal absorption would be promoted and more uniform and higher blood concentrations of its free and conjugated fractions would be assured. The coadministration of 1 molar acetic acid led to acetylation of its amino groups in vivo with consequent reduction of its toxicity.

The object of these experiments was to determine the comparative therapeutic efficacy of sulfanilamide and an acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride in experimental beta hemolytic streptococcus pericarditis in the rabbit. Sulfanilamide was selected as the standard antistreptococcic agent among the present members of the sulfonamide group.

PREPARATION, PROPERTIES AND MICROCHEMICAL ANALYSES OF  
4,4'-DIAMINODIPHENYLSULFONE AND THE ACETYLATED  
DERIVATIVE OF ITS HYDROCHLORIDE

The compound 4,4'-diaminodiphenylsulfone was originally prepared by Fromm and Wittmann<sup>7</sup> by a reduction of the corresponding dinitro compound that was obtained by oxidation of 4,4'-diaminodiphenylsulfide.

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5. Buttle, G. A. H.; Dewing, T.; Foster, G. E.; Gray, W. H.; Smith, S., and Stephenson, D.: *Biochem. J.* **32**:1101, 1938.

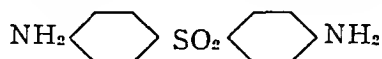
6. Roblin, R. O., Jr.; Williams, J. H., and Anderson, G. W.: *J. Am. Chem. Soc.* **63**:1930, 1941.

7. Fromm, E., and Wittmann, H.: *Ber. d. deutsch. chem. Gesellsch.* **41**:2264, 1908.

Raiziss and co-workers<sup>8</sup> have described several improved methods for its preparation, one of which consists in oxidation of 4,4'-diaminodiphenylsulfide (thioaniline) with protection of the amino groups by acetylation and subsequent hydrolysis. By this procedure a yield of 80 per cent of a fairly pure product was obtained, which was further purified by recrystallization from 50 per cent alcohol.

The preparation used in these experiments was prepared from pure 4-nitro-4'-aminodiphenylsulfide as a starting material by oxidation, and then recrystallized several times for purity.<sup>9</sup>

It is a crystalline solid compound, consisting of almost colorless long rectangular plates. It possesses the following structural formula:



It is sparingly soluble in water at room temperature, only to the extent of 0.01 per cent, and is but slightly more soluble in hot water, to the extent of 0.05 per cent. Its melting point is 176 C. Certain physical and chemical constants, found by microchemical determinations and run in duplicate, averaged as follows: carbon, 57.87 per cent; moisture content, 1.30 per cent; nitrogen, 11.00 per cent; hydrogen, 5.16 per cent; sulfur, 12.91 per cent. The samples left no ash. These determinations were carried out by Dr. Carl Tiedcke, Laboratory of Microchemistry, New York. The calculated percentages for nitrogen and sulfur were, respectively, 11.29 and 12.99. Thus, the found and the calculated percentages for nitrogen and sulfur were in good agreement, indicating that the prepared compound was of high purity.

The product was converted into a hydrochloride by the addition of 1.0 cc. of 1 molar hydrochloric acid to 0.1 Gm. of drug. Chemical analysis of the compound for the hydrochloride radical showed that the reaction was completed within thirty minutes.

#### DETOXIFICATION OF 4,4'-DIAMINODIPHENYLSULFONE

The term "detoxification" as employed in this investigation implies either total abolition or reduction of the toxicity of a substance as the result of certain changes which it undergoes in the animal body. Acetylation is concerned in the detoxification of amino groups and represents a mechanism by which the animal organism protects itself against foreign toxic substances.<sup>10</sup> A well known example of this is the acetylation of

8. Raiziss, G. W.; Clemence, L. W.; Severac, M., and Moetsch, J. C.: J. Am. Chem. Soc. **61**:2763, 1939.

9. The preparation was furnished by Dr. G. W. Raiziss, Dermatological Research Laboratories, Division of Abbott Laboratories, Philadelphia.

10. Sherwin, C. P.: Physiol. Rev. **2**:238, 1922. Stekol, J. A.: Ann. Rev. Biochem. **10**:265, 1941.

the amino group of p-aminobenzoic acid. Klein and Harris<sup>11</sup> found that the acetylation of sulfanilamide in vitro by slices of rabbit and human liver is determined by the rate of the production of acetate by the tissues. The addition of acetate or of its precursors in the tissues increases the degree of acetylation of sulfanilamide. Acetylation occurs only in the presence of intact and functioning liver cells and is not reversible. James<sup>12</sup> confirmed the observations of Klein and Harris and noted an increased excretion of acetylsulfanilamide in the urine of mice when sodium acetate was fed with sulfanilamide.

With the object of reducing the acute toxicity of 4,4'-diaminodiphenylsulfone, experiments were carried out with ascorbic acid, nicotinic acid, dextrose and acetic acid and its precursors in vivo, namely, lactic and pyruvic acids, and with cystine, glycine and other substances. After many trials, 1 molar acetic acid was selected as the most satisfactory agent for this purpose. It provides directly the acetyl radical which replaces in part or in full the amino groups. The administration of acetic acid with the sulfone compound definitely protects the rabbit against the acute and chronic toxic effects of the sulfone compound by lessening their frequency and severity. At the same time it does not materially affect the curative value of the drug. It appears that acetylation of the sulfone hydrochloride serves as a detoxicating mechanism. It is also of interest to observe in different infected animals that there is considerable variation in the acetylation of the drug even in the presence of added 1 molar acetic acid.

#### MATERIAL AND METHODS

*Rabbits.*—Throughout this series of experiments, normal adult male rabbits, 6 to 7 pounds (2.5 to 3 Kg.) in weight, of the breed known as the New Zealand white, were used. It was found that the weight, sex and breed were significant in that the period of survival of the untreated control animals and the incidence of toxic reactions in the treated groups were somewhat dependent on these factors, fluctuating especially with the weight. Each animal was maintained under constant dietary conditions and housed in the same individual cage for one week or more prior to operation. The animals proved to be uniformly susceptible to intrapericardial infection with the beta hemolytic streptococcus.

*The Strain of the Beta Hemolytic Streptococcus and the Size of the Inoculum Introduced into the Pericardial Sac.*—The strain of the beta hemolytic streptococcus originally isolated by Gay from the pleural cavity in a fatal case of human empyema was employed in this investigation. After being passed through the pleural cavities of many generations of animals, its pedigreed stock culture, designated H, has attained and retained a standard virulence for rabbits. The strain belongs to Lancefield's group A and is smooth both as to virulence and as to colony form.

11. Klein, J. R., and Harris, J. S.: J. Biol. Chem. **124**:613, 1938.

12. James, G. V.: Biochem. J. **33**:1688, 1939; **34**:633, 1940.

Its virulence was preserved by desiccation of the cultures and storage in the frozen-dried state with suppression of their metabolic processes:<sup>13</sup> From a blood agar plate culture, representative hemolytic colonies were inoculated into beef infusion broth and incubated at 37 C. for twenty-four hours. Subcultures in this broth were stored in the cold for twenty-four hours. Concentrated suspensions of bacterial cells from the broth cultures incubated for fifteen hours were then frozen and desiccated over phosphorus pentoxide. The tubes of dried cultures were sealed and stored in the icebox at 3 to 6 C. Their virulence was preserved in this fashion for at least six months.

Three days before the production of the suppurative pericarditis, 0.5 cc. of veal infusion broth was added by pipet to a tube of dried culture, and 0.2 cc. of this suspension was transferred to veal infusion broth. Transplants were made in this medium twice daily for three successive days. From a five hour subculture, a 4 mm. platinum loopful was suspended in 5.0 cc. of tryptose broth, and from this 1:10, 1:100 and 1:1,000 dilutions were made in tryptose broth. Duplicate plates were made with 0.12 cc. of each of the dilutions in 10 cc. of freshly melted tryptose agar, incubated at 37.5 C. for forty-eight hours and the colonies counted with a colony counter. Simultaneously with these platings, 0.12 cc. of each dilution was inoculated intrapericardially into a series of 4 rabbits to determine the lowest number of micro-organisms capable of producing a fatal infection within three to five days. This was generally found to be between 3,000 and 5,000 colonies per 0.12 cc., each colony representing a single bacterium or an aggregate of bacterial cells. This procedure was repeated with each group of controls and of treated animals. Within certain limits it was found that the size of the infective dose was not definitely related to the duration of the pericarditis; rather, the duration was a function of the breed and sex, and especially of the weight of the animals.

*The Production of Experimental Beta Hemolytic Streptococcus Pericarditis in the Rabbit by an Extrapleural Route and in a Single Stage Operation.*—Despite the delicacy of the pericardial membranes of the rabbit, the pericardial cavity can be exposed without entrance into either pleural sac, because of the widely separated and complete mediastinal partitions.

After the usual preparations of the anterior thoracic wall, the skin and subcutaneous tissue over the lower third of the sternum were incised in the midline under preliminary anesthesia induced with soluble pentobarbital followed by light inhalation of ether to induce satisfactory relaxation. The underlying fascia and muscles were cleared away from the ribs on both sides of the sternum. With a small bone-cutting forceps, the sternum was resected transversely between the seventh and eighth ribs, and then these ribs were divided as close as possible to the sternal margins to avoid injury to the internal mammary arteries. Their injury led to troublesome and even fatal hemorrhage. The mammary vessels are situated at some distance from the sternum and, if necessary, can usually be sufficiently retracted so that their ligature is unnecessary. Experience also showed that it was important not to sever the ribs at a point too distant from their sternal attachments; otherwise fatal pneumothorax might follow from injury to the pleural membranes. The approach described provides a satisfactory exposure of the pericardial sac.

After the removal of the resected piece of sternum, the beating heart was easily visible through the transparent pericardium. Any fat over the parietal layer

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13. Wadsworth, A. B.: Standard Methods of the Division of Laboratories and Research of the New York State Department of Health, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 57.

obscuring the view of the heart was carefully dissected away. The parietal pericardium was seized with a fine mouse-toothed forceps or hemostat, and the infecting dose of organisms, contained in 0.12 cc. of nutrient broth warmed to body temperature, was injected into the sac by means of a tuberculin syringe with an attached no. 26 hypodermic needle, with the usual precautions for asepsis. Care was taken not to injure the coronary vessels or the myocardium. The needle was then withdrawn, the puncture wound closing rapidly without apparent leakage of the inoculum into the adjacent tissues. If desired, the puncture wound of the pericardium may be securely ligated beneath the tip of a hemostat. Replacement of the resected piece of sternum was not necessary. The incision was sutured layer by layer. The wound was dressed with sterile gauze, and the animals recovered rapidly from the operation.

Another satisfactory approach to the pericardium is through a small opening on the left or the right side close to the sternum. The third rib is exposed, and after 1 to 2 cm. have been resected, the intercostal muscles and membranes between the lower border of the second and the upper border of the fourth rib are cleared away.

Under the perfected conditions of experimentation and without resorting to any elaborate operative technic, uniformly fatal suppurative pericarditis was reproduced with absolute regularity within a narrowly defined period.

*Blood Cultures.*—The blood cultures were taken immediately prior to the administration of the drugs and daily in the untreated group, the group treated with the sulfone compound and the group treated with sulfanilamide until recovery or death. From the marginal vein of an ear 1 cc. of blood was withdrawn with an accurately graduated tuberculin syringe, and tryptose broth dilutions of the blood were immediately plated in cooled Bacto blood agar base and incubated at 37.5 C. for at least seventy-two hours. The colony counts represented the number of single cocci and aggregations of beta hemolytic streptococci per cubic centimeter of circulating blood; they yielded an approximate comparison of the different groups of animals with respect to the bacteremia present at various stages.

*Chemical Study of Blood Specimens.*—Blood was obtained routinely two hours after the administration of sulfanilamide and after the administration of the sulfone compound with concomitant administration of 1 molar solutions of acetic and hydrochloric acid. The free and the combined sulfanilamide and the acetylated derivative of the 4,4'-diaminodiphenylsulfone hydrochloride in its free and in its conjugated state in the blood were determined by the method of Bratton and Marshall.<sup>14</sup> The sulfone compound yielded a purplish red reaction similar to that of sulfanilamide by diazotization and by coupling of the resulting diazo compound. The purplish red dye compound was estimated colorimetrically with the Klett-Summerson photoelectric cell colorimeter. The plasma carbon dioxide-combining power was estimated by the method of Van Slyke and the serum chlorides by the micromethod of Saiffer and Kornblum.<sup>15</sup>

*Blood Counts.*—Since the leukocytes of the blood of normal rabbits are subject to hourly variations, individual white cell counts were performed daily during the same period, namely, from 9 to 10 a. m., for several days preceding operation, to establish the general normal leukocyte trend for each animal. Two cover slip

14. Bratton, A. C., and Marshall, E. K. Jr.: *J. Biol. Chem.* **128**:537, 1939.

15. Saiffer, A., and Kornblum, M.: *J. Biol. Chem.* **36**:112, 1935.

blood films were prepared with Wright's stain at the same time, and 300 leukocytes were counted in each smear. The hemoglobin estimations were carried out by the Newcomer method.

The total number of leukocytes per cubic millimeter in the normal animals ranged from 5,200 to 14,300, with a mean average of 9,700. The average differential percentages were as follows: neutrophils (amphophils), 36; lymphocytes, 59; large mononuclear cells, 2; basophils, 2; eosinophils, 1. The average total red cell count was found to be 5,280,000 cells per cubic millimeter, and the hemoglobin content ranged around 85 per cent.

*Histologic Studies.*—Many representative blocks of myocardium and overlying visceral pericardium and of lungs, spleen, liver, kidney, bone marrow and brain were sectioned for microscopic study and stained with hematoxylin-erythrosin, Van Gieson's stain, Masson's trichrome *lichtgrün* stain, and the Gram-Weigert stain for bacteria. In addition, films of the pericardial exudate and sections of the inflamed pericardium were stained for fibrin by the method of Weigert and for oxidizing ferments by the method of Schultze.

#### ADMINISTRATION AND DOSAGE OF DRUGS

Altogether, 250 rabbits were infected intrapericardially and divided into five different groups, one of which served as a control. In the various series, treatment was commenced at four, twelve, twenty-four, and thirty-six hours after the injection of the micro-organisms into the pericardial sac. These groups were designated respectively as A, B, C and D.

Of importance for successful therapy are such factors as the virulence of the strain and the type of streptococcus, the lapse of time between infection and treatment, the maximum tolerated dose in grams per kilogram of body weight, the total dose of drug administered, the intervals between doses, the maintenance of an adequate and constant concentration of the drugs in the blood and tissues, the length of the period of therapy and the absence of severe untoward acute and chronic reactions. The dose of each drug employed in the various groups was computed on the basis of data relative to these factors obtained in preliminary trial experiments in normal and in infected rabbits.

Group A was treated with sulfanilamide, the administration of the drug starting four hours after the production of pericarditis. The drug was prepared in a 1 per cent aqueous solution, which was introduced directly into the stomach by means of a hard rubber catheter in amounts of 3.0 cc. every six hours during the life of the animal. The total daily dose was 1.2 Gm. and was within the limits of the maximum tolerated dose of 1.0 Gm. per kilogram found for the normal rabbit by Trefouël and co-workers.<sup>16</sup> The administration of the drug in aqueous solution rather than as a solid in gelatin capsules not only assured more constant absorption from the intestinal tract and more adequate concentration

16. Fourneau, E.; Tréfouël, J.; Nitti, F., and Bovet, D.: Bull. Acad. de méd. 118:210, 1937.



in the blood, despite the relatively low aqueous solubility of the compound, but aided in providing an adequate fluid intake. The total blood sulfanilamide level fluctuated during treatment from 7.4 to 19.2 mg. per hundred cubic centimeters, of which 20 to 51 per cent was conjugated. It was observed that the conjugation of the drug varied greatly with different animals and that its rate and trend were characteristic for a given animal.

It is well known that the rabbit may manifest an irregular toxic response to sulfanilamide. However, this series exhibited no severe untoward symptoms with the dosage indicated in the foregoing paragraph. It should also be noted that the dose in terms of kilograms of body weight was far in excess of that ordinarily employed in human therapy and that treatment was commenced eight hours earlier than in the twelve hour group B, treated with the acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride.

The schedule of dosage with the sulfone hydrochloride in the initial two critical days of infection was varied in the different groups. It was found that the less severely ill twelve hour interval group B tolerated the compound better than groups C and D. Thus, the dosage was adjusted to the severity of the pericardial infection and its resultant bilateral empyema and bacteremia. Group B received 2.4 Gm. in the first day and 2.0 Gm. in the second day in four divided doses, one being given every six hours. Groups C and D were given 2.0 Gm. each in the first day, and 1.5 Gm. and 1.0 Gm., respectively, in the second day of treatment in four divided doses at six hour intervals. A maintenance dose of 1.2 Gm. was given daily in three divided doses at eight hour intervals for ten successive days to each animal in groups B, C and D until recovery or death.

The drug suspended in 1 molar solution of hydrochloric acid was forced into the stomach through a hard rubber catheter, and acetic acid and physiologic solution of sodium chloride were then given immediately in the order named. The latter was discontinued after a few days, when the animals resumed eating and drinking. For each 0.1 Gm. of drug, 1.0 cc. each of 1 molar hydrochloric acid and 1 molar solution of acetic acid was given. Physiologic solution of sodium chloride was administered in 15 cc. amounts with the doses to maintain an adequate water-salt balance and to guard against the development of dehydration and urolithiasis.

Raiziss and co-workers<sup>3</sup> have shown that 0.3 to 0.5 Gm. of 4,4'-diaminodiphenylsulfone per kilogram was the maximum tolerated dose of normal rabbits. Single doses as high as 1.5 Gm. per kilogram, administered concomitantly with 1 molar solutions of hydrochloric and acetic acids, were readily tolerated by normal rabbits without acid or injurious drug effects. The doses of acetylated derivative of 4,4'-diamino-

diphenylsulfone hydrochloride in the infected animals were well within the single maximum tolerated dose for normal rabbits and, although relatively high, were well borne.

The drug was rapidly absorbed from the intestinal tract and was present in estimable amounts in the blood stream within half an hour after oral administration. Maximum concentrations were attained within two hours and maintained at peak levels for another two to three hours. Administration of the drug during the first two days of treatment led to a total blood sulfone concentration of 10 to 21 mg. per hundred cubic centimeters, of which 15 to 63 per cent was present as the active free amino fraction. Despite the simultaneous feeding of acetic acid providing an ample supply of acetyl radical, the rate and the degree of conjugation varied in different rabbits. However, conjugation was characteristically constant for the individual animal, in sharp contrast to the irregularities observed in the case of 4,4'-diaminodiphenylsulfone. The adequate and constant blood concentrations following the administration of the sulfone hydrochloride were attributed to the increased water solubility of the hydrochloride derivative, promoting more uniform intestinal absorption.

The therapeutic effects of the drug could not be consistently correlated with the total blood concentrations. However, there appeared to be a fairly consistent correlation between therapeutic effectiveness and the level of the free, active fraction. Thus, the most satisfactory results were obtained with a concentration of the free drug of between 4 and 8 mg. per hundred cubic centimeters.

The variations in the carbon dioxide capacity of the blood plasma were within or slightly below normal limits. In no instance did the carbon dioxide-combining power fall below 40 volumes per cent. The hydrogen ion concentration of the blood plasma remained at neutrality. Hyperventilation and other evidences of acidosis were not detected. The serum chlorides were normal.

#### TOXICITY

A group of 10 normal adult rabbits of the same breed employed in the experiments received the identical doses of acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride at the same intervals and for the same period as the twelve hour set of treated animals. They were observed daily for four weeks for evidence of acute and chronic intoxication. None of the animals exhibited any untoward effects, such as cyanosis, dyspnea, anorexia, diarrhea or symptoms and signs referable to the nervous system.

Semiweekly examination of the peripheral blood revealed in the third week the development of early anemia, which progressed slightly, so that at the end of the period of observation the red cell count fell between 3,500,000 to 3,000,000 per cubic millimeter and the hemoglobin content between 65 and 57 per cent. These changes were accompanied by a rise

of the reticulocytes in the peripheral blood to 2 and 8 per cent. The leukocyte counts fluctuated within physiologic limits or were slightly raised above normal, but in no instance was there observed a condition comparable to agranulocytosis. Microscopic examination of the bone marrow after four weeks showed early inhibition of erythropoiesis. From their appearance, the animals were in good health. Thus, apart from a slight loss of weight and a progressive low grade anemia, this group showed no toxic reactions.

Despite the concomitant administration of 1 molar acetic acid, the treatment of all infected animals was attended by an incidence of 11.7 per cent toxic reactions. These were more frequent and more severe in the thirty-six hour set than in the others. The earliest toxic manifestation noted was cyanosis of the lips and nasal mucous membranes not associated with an increased respiratory rate. With progressive severity, variably severe dyspnea and muscular weakness of the limbs with stumbling gait and uncoordinated movements developed. However, these reactions were generally transient, lasting but one to two days and disappearing completely except in a small proportion of fatal cases.

Within two to three days after infection, the red cell counts and the hemoglobin values of the treated and the control group dropped sharply to 3,000,000 and 3,500,000 per cubic millimeter and 55 to 47 per cent. With recovery, regeneration of the red blood cells and hemoglobin, accompanied by increased numbers of reticulocytes in the peripheral blood, was rapid. In contrast, the red cell count and the hemoglobin value continued to fall sharply in the untreated group, reaching levels as low as 2,100,000 per cubic millimeter and 38 per cent shortly before death. The acute hemolytic anemia occurring in both sets is attributed essentially to the direct action of the beta hemolytic streptococci on the bone marrow and circulating erythrocytes rather than to the drug.

#### RESULTS OF TREATMENT AND COMMENT

An important requisite for therapeutic investigations on acutely infected animals is that the experimental infection should pursue a regular course, preferably with a fatal termination, in a narrowly defined period. This object was successfully attained by the constant death of all the control animals within three to five days after the production of the pericardial infection. Survival with cure of the suppurative pericarditis by resolution, by fibrosis or by both, prolongation of life, and death were adopted as the criteria for the assessment of the value of the therapy.

The most significant observation in this study was that of the comparatively high antistreptococcal activity and efficacy of the acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride. Of the 50 animals comprising the twelve hour interval treated group B, there

are 49, or 98 per cent, cured survivors. The one death occurred on the twenty-third day of treatment. In group C there are 68 per cent cured survivors, with a prolongation of life of from eleven to twenty-two days (average, seventeen and eight-tenths days) in the remainder. The compound is clearly not very effective in the thirty-six hour interval treated group D, in which the cured survivors numbered only 10 per cent. The duration of life in this group is considerably decreased, ranging from six to sixteen days and averaging but nine and two-tenths days.

The results clearly show that the range of effective doses is not wide and that treatment to be most successful must be initiated at an early stage of the pericarditis, namely, twelve to twenty-four hours after the introduction of the organisms into the pericardial sac. Although the separate groups treated with the sulfone compound are not as large as those used by investigators of the effect of this compound on experimental streptococcic infections in mice, the results are significant in consideration of the absolute constancy of death within three to five days in the untreated control group.

It appears that the explanation of the high death rate in the severely ill group D is to be sought in failure of immune response and in depression of the activity of the host's phagocytic cells together with the relatively lower total dose administered in the critical first two days of the fulminating pericarditis. The individual variation in resistance is also a significant factor in modifying results despite the fact that the different groups of animals were of the same breed, weight and sex and were infected with approximately the same inoculum of the same culture under identical conditions of experimentation. Another factor responsible for the low percentage of survivors in this group may have been the presence of still undemonstrated substances in the pericardial exudate inhibiting the action of the sulfone compound.

Loewenthal<sup>17</sup> brought forward evidence to show that sulfanilamide and antistreptococcic serum, each acting in a different but complementary manner, exert a synergic and enhanced therapeutic action in mice infected with streptococci. A potent and homologous antistreptococcic serum and the sulfone compound investigated or one of proved superiority administered simultaneously might have acted in a similar manner with increased effectiveness in the severely ill animals of group D, leading to a higher rate of cures.

Figure 1 definitely shows that sulfanilamide failed to cure a single one of the animals treated and prolonged life for but one to seven days (average, three and eight-tenths days) beyond the control period despite the commencement of treatment only four hours after the production of the pericarditis and despite the adequate dosage of the drug. The failure

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17. Loewenthal, H.: *Lancet* 1:197, 1939.

of sulfanilamide to influence decisively the natural course of the pericarditis may be attributed to its lower chemotherapeutic activity in comparison with the sulfone compound, the severity of the infection and, perhaps, to inhibition by unknown substances<sup>18</sup> in the early stage of the pericarditis and union with proteins<sup>19</sup> leading to inactivation. It may be deduced that sulfanilamide is without marked beneficial action on experimental beta hemolytic streptococcus pericarditis in the rabbit.

The first signs of recovery in animals treated with the sulfone compound are a return of appetite with a resumption of eating and an increased intake of fluid on the first to third day after the commencement of treatment, accompanied by a rapid fall in temperature. The temperature of an acutely infected animal is apparently less susceptible to

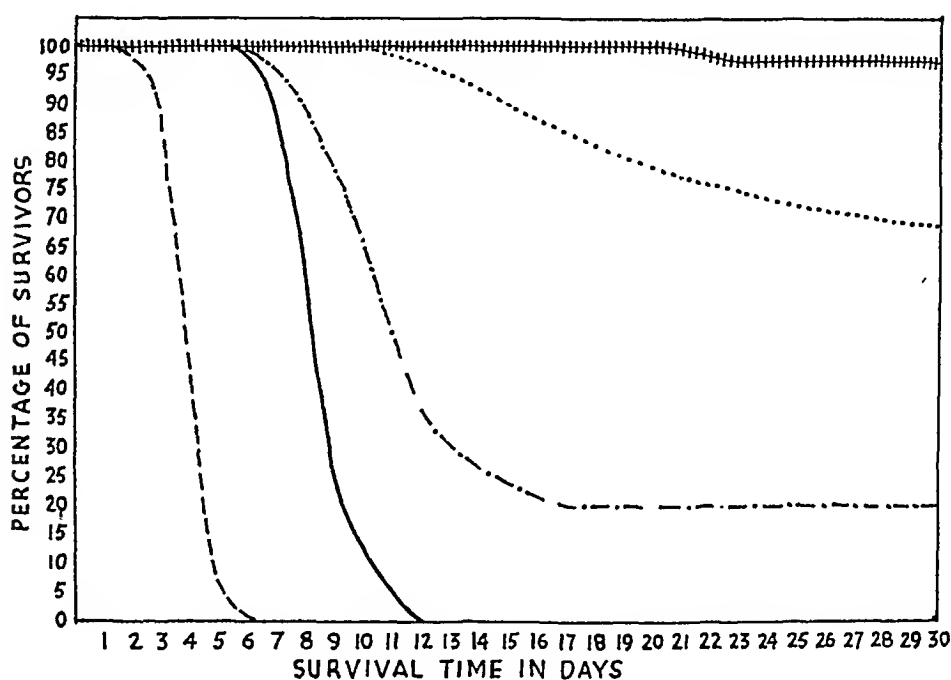


Fig. 1.—Effects of sulfanilamide and acetylated 4,4'-diaminodiphenylsulfone hydrochloride in experimental beta hemolytic streptococcus pericarditis in rabbits. The percentage of survivors in each group of 50 animals is shown by a curve: — — — —, control untreated group; —————, sulfanilamide-treated group, in which treatment was started four hours after the production of pericarditis; + + + +, group in which treatment with the sulfone compound was started twelve hours after the production of pericarditis; . . . . ., group in which treatment with the sulfone compound was started twenty-four hours after the production of pericarditis; . — . — . —, group in which treatment with the sulfone compound was started thirty-six hours after the production of pericarditis.

environmental influences than that of a normal one. Therefore, a fall is significant when correlated with other evidence.

18. Marshall, E. K., Jr.: *Ann. Rev. Physiol.* **3**:652, 1941.

19. Davis, B. D.: *Science* **95**:78, 1942.

In the absence of extensive streptococcic pneumonia the respiratory excursions do not rise above the normal rate of 30 per minute. In the animals that recovered the blood stream was rapidly cleared of circulating micro-organisms by 1.5 to 2.0 Gm. or more of the sulfone compound, as a rule, within twenty-four to forty-eight hours after the initial demonstration of the bacteremia. The rapid loss of body weight during the period of active infection is principally dependent on the local and systemic effects of the micro-organisms and, in a lesser measure, on the refusal of the animals to take food. Once recovery sets in, the gain in weight is rapid. It is of interest to note that animals dying two weeks or later after the commencement of treatment, despite an adequate daily maintenance dose, reveal no conspicuous symptoms.

Under the conditions of experimentation the curative effect of the sulfone compound is complete in the sense that the micro-organisms are not recoverable by culture from the tissues of the cured rabbits and that the pericarditis is completely healed by resolution, by fibrosis or by both.

The therapeutic efficacy of the acetylated derivative of the 4,4'-diaminodiphenylsulfone hydrochloride is apparently related to the presence of its two active paraamino groups, which are evidently made available by deacetylation in vivo in body fluids and tissues by reduction through hydrolysis of the acetylated derivative. The therapeutic effectiveness is governed also by the following factors: (1) the rapidity of absorption of the sulfone hydrochloride from the intestinal tract, evidently dependent on its solubility in water and lipids or on other mechanisms; (2) the speed of development and constant maintenance of an adequate and effective concentration of the agent in the blood with a suitable ratio of the free, unconjugated and acetylated fractions of the drug; (3) the rate and the degree of diffusion into the body tissues and fluids; (4) the elimination of the drug; (5) its toxicity to the host tissues and organs, and (6) its concentration within the bacterial cells, which is probably of the same order as that of the milieu. Most of these factors have already been discussed in other sections.

It is not certain that sulfur is a necessary element in a bacterial chemotherapeutic agent. Sulfanilamide contains sulfur in the form of the sulfonamide group ( $\text{SO}_2\text{NH}_2$ ). Similarly, the acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride contains sulfur, but whether or not the sulfur atom is indispensable for its therapeutic properties remains to be established. Nevertheless, the fact remains that no compound therapeutically effective in streptococcic infections has yet been synthesized which does not contain sulfur in some form.

The precise mode of action of the sulfone compound remains a problem for future investigation. It is apparently bacteriostatic in vivo and perhaps even bactericidal to a certain extent.

Ultimate recovery is determined by the inhibition of multiplication of the streptococci through the bacteriostatic action of the sulfone com-

pound aided by the phagocytic activity of the mobilized neutrophils and mononuclears. There is no proof that the drug stimulates the production of phagocytes and antibodies or promotes phagocytosis. The neutrophil and macrophage reaction is a constant accompaniment of recovery.

Microscopic observations on the animals put to death clearly show that the mobilization of the mononuclears in the visceral and parietal pericardium so significant in healing appears to be the result of proliferation of the local tissue histiocytes. Histologic examination affords no support for the assumption that the cells of the reticuloendothelial system cooperate in controlling the infections.

Are these results of significance for the treatment of hemolytic streptococcus infection in man? Since the dose employed in the rabbit multiplied weight for weight represents an enormous dose of the acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride for man, the question naturally arises as to whether any inferences at all may be drawn from these experiments in respect to the use of the compound against human infections. It should, however, be noted that the experimental pericarditis in the rabbit is far more severe than spontaneous suppurative pericarditis in man. All untreated control animals show a mortality rate of 100 per cent, with death occurring within three to five days, whereas the death rate for suppurative pericarditis in man is stated to be between 30 and 50 per cent<sup>20</sup> and the process is of longer duration. In point of fact, pericarditis in the rabbit is rather comparable to that in the rare patients who from the outset are overwhelmed by the invading streptococci.

Another great difficulty lies in the transfer of results from an animal species to man, since different host species may vary either qualitatively or quantitatively in their behavior both to the pathogenic micro-organisms and to the drugs. Nevertheless, despite these limitations, the essential basis for the clinical use of new synthetic drugs must continue to be in their performance under experimental conditions.

PATHOLOGIC OBSERVATIONS ON THE EVOLUTION OF EXPERIMENTAL PERICARDITIS IN THE UNTREATED ANIMALS  
AND ON THE INFLUENCE OF SULFANILAMIDE AND  
ACETYLATED 4,4'-DIAMINODIPHENYLSULFONE  
HYDROCHLORIDE ON ITS COURSE

Controls and three sets of animals treated with the sulfone compound twelve and twenty-four hours and with sulfanilamide four hours after the inception of the infection were put to death serially in pairs at intervals of twelve, twenty-four and forty-eight hours and four, seven, fourteen and

20. Shipley, A. M., and Winslow, N.: *Arch. Surg.* **31**:375, 1935. Billings, A. E.: *S. Clin. North America* **12**:1517, 1932.

twenty-one days beyond the period in which death naturally occurred in the untreated animals. A study of the changes in the pericardial sac and other important organs was undertaken.

*Evolution of the Pericarditis and Associated Lesions in the Control Animals.*—Twelve hours after infection, changes in the pericardial sac are already discernible. The pericardial membranes appear dull and are covered with a delicate film of grayish exudate. Blood agar plates yield about five times as many single and chained cocci as were originally injected into the sac. The pleural cavities are still macroscopically normal and culturally sterile. The lungs are free of inflammatory consolidation. The other viscera are not remarkable on gross inspection.

At the twenty-four hour stage the pericardial sac contains 1.0 to 1.5 cc. of thin, yellowish white fibrinopurulent exudate. The pleural cavities and the lungs are usually still free of infection at this time.

At forty-eight hours and onward to the time of death the amount of fibrinopurulent exudate in the pericardial sac increases rapidly and markedly, so that at death, three to five days from the inception of the pericarditis, the sac is greatly distended by 6 to 10 cc. of thick, creamy, greenish or brownish gray fibrinopurulent effusion as measured with a syringe after agitation with a few cubic centimeters of sterile physiologic solution of sodium chloride. The pericardium is covered with opaque, grayish white, friable exudate from 1 to 3 mm. in depth, which strips without difficulty (fig. 2 A). Serofibrinous pleural infusion is constantly present at the forty-eight hour stage, later becoming frankly purulent and bilateral, and associated with multiple areas of red to grayish red hepatization in the lung. The empyema following the pericarditis does not appear to involve directly the parenchyma of the lung.

At death the lymph nodes draining the pericardium and situated at the base of the heart appear swollen and moist. The myocardium is the seat of diffuse severe parenchymatous degeneration. The cardiac valves are free of vegetations. There are widespread edema and suppuration of the thoracic wall, associated with extensive formation of tissue sloughs related to the puncture of the pericardial sac and injection of the infecting dose. The peritoneal cavity is not invaded by bacteria from either the pericardial or the pleural cavities. The spleen appears enlarged, friable and dark red, with extensive and marked obliteration of its normal landmarks. The stomach is acutely dilated. The liver and the kidneys show severe parenchymatous degeneration.

The pericardium of the normal rabbit contains but few mononuclear cells. It is externally lined with a single layer of flat serosal cells. Microscopic examination of the pericardium at the twelve hour stage of infection reveals universal vascular congestion and edema of the subserous layers associated with slight deposition of fibrin and formation



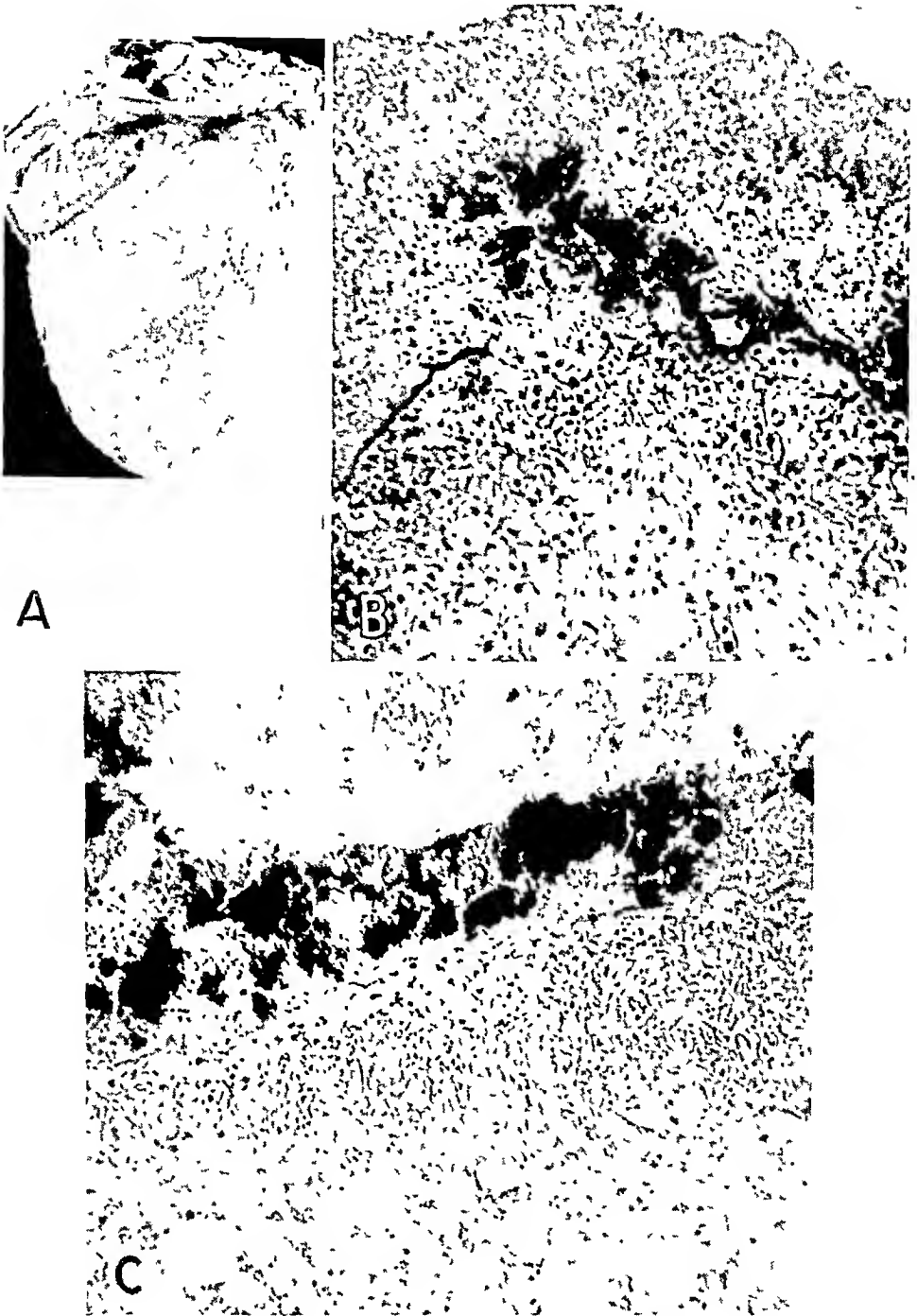


Fig. 2.—*A*, anterior view of the heart of an untreated rabbit which died on the fourth day, showing exudate over the visceral pericardium.

*B*, photomicrograph of a section of the heart shown in *A*, exhibiting heavy necrotic fibrinopurulent exudate over the visceral pericardium and edema and degeneration of the superficial myocardium; hematoxylin and erythrosin;  $\times 210$ .

*C*, Photomicrograph of a section of the heart of a sulfanilamide-treated rabbit dying seven days after the production of the pericarditis. Note the myriads of gram-positive streptococci in the visceral pericardium and the characteristic degeneration and necrosis of the cellular exudate; Gram-Weigert stain for bacteria;  $\times 230$ .

of cellular foci consisting almost entirely of neutrophils as observed in the oxidase-stained preparations. At this stage, sections stained for bacteria by the Gram-Weigert method show but a relatively small number of cocci, for the most part extracellular. In the successive series there is an increasingly heavy deposit of fibrin and leukocytes with the neutrophil as the dominant cell type. This is accompanied by interstitial edema and degeneration and necrosis of the superficial muscle fibers of the myocardium. The leukocytic exudate (fig. 2B) from the second day onward is distinguished by progressive degeneration and necrosis attended by steady increase in the number of cocci. Myriads of streptococci are present at the time of natural death, but they are very largely extracellular and gram-positive in their staining reaction. Phagocytosis is not common, the ingested cocci appearing, as a rule, involutioned and, at times, in short chains. No attempt was made to stain the capsules of the streptococci in the tissues.

*Evolution of the Pericarditis and Associated Lesions in Rabbits Treated with Sulfanilamide.*—In degree and extent the pericarditis and the local lesions accompanying it are indistinguishable on macroscopic examination from those observed in the untreated pairs of animals. Histologically, the pericardial exudate differs from that in the controls by a slight shift in its cellular composition toward the mononuclears, which still constitute the minority group of cells, and by a slight increase in phagocytosis. The micro-organisms are unlimited in number at death and are chiefly extracellular (fig. 2C).

*Evolution of the Pericarditis and Associated Lesions in Rabbits Treated with the Acetylated Derivative of 4,4'-Diaminodiphenylsulfone Hydrochloride.*—A comparison of the suppurative pericarditis and its local and distant complications in the sulfone-treated, the sulfanilamide-treated and the control groups clearly reveals that the sulfone compound not only profoundly alters the course and spread of the pericarditis but the histologic pattern of the infection as well.

These changes are already evident in rabbits of the twelve hour treated group put to death after twenty-four hours of treatment. Post-mortem examination reveals no gross evidence of fluid exudate in the pericardial sac except for a few drops of dark blood-stained sterile fluid. The pericardium is restored to its normal state except for a few small irregular opaque adherent areas of grayish white exudate undergoing organization. There is no evidence of spread of the pericardial infection into the mediastinum or into the pleural cavities. The lungs are free of consolidation. The intestines are not distended. The liver, the spleen and the kidneys are extraordinarily free of pathologic changes. Both the kidneys and the bladder show no deposition of crystals or formation of concrements at this stage or later. The essential healing process in

this group of animals as revealed in the successive stages is that of resolution with or without fibrosis (fig. 3).

The twenty-four hour interval pairs of animals put to death after one day of treatment show from 0.7 to 1.0 cc. of thin straw-colored fluid effusion within the pericardial sac associated with multiple irregular deposits of exudate in an early stage of organization and a few small gray to pinkish yellow semigelatinous masses loosely adherent to the visceral and the parietal pericardium. Frank purulent exudate is absent. Macroscopic examination of the lungs reveals no inflammatory consolidation. The animals put to death after four days contain no measurable fluid exudate within their pericardial sacs.



Fig. 3.—Photomicrograph of a section of the heart of a rabbit on the twenty-first day after a twelve day course of treatment with acetylated 4,4'-diaminodiphenyl-sulfone hydrochloride commenced twelve hours after induction of the pericarditis. Note healing by resolution and by fibrosis. Hematoxylin and erythrosin;  $\times 380$ .

In comparison with the pericardial exudate in the control and the sulfanilamide-treated animals, that in the twelve hour and the twenty-four hour interval sulfone-treated animals presents a distinct change in its cellular composition as a result of treatment. After twenty-four hours of treatment the neutrophils are present in large numbers, distinctly predominating over the macrophages. Both (fig. 4 *A*), especially the macrophages, are actively phagocytic for bacteria, which are strikingly reduced and chiefly intracellular. There is a rough parallelism between the intensity of the mobilization of the neutrophils and mono-

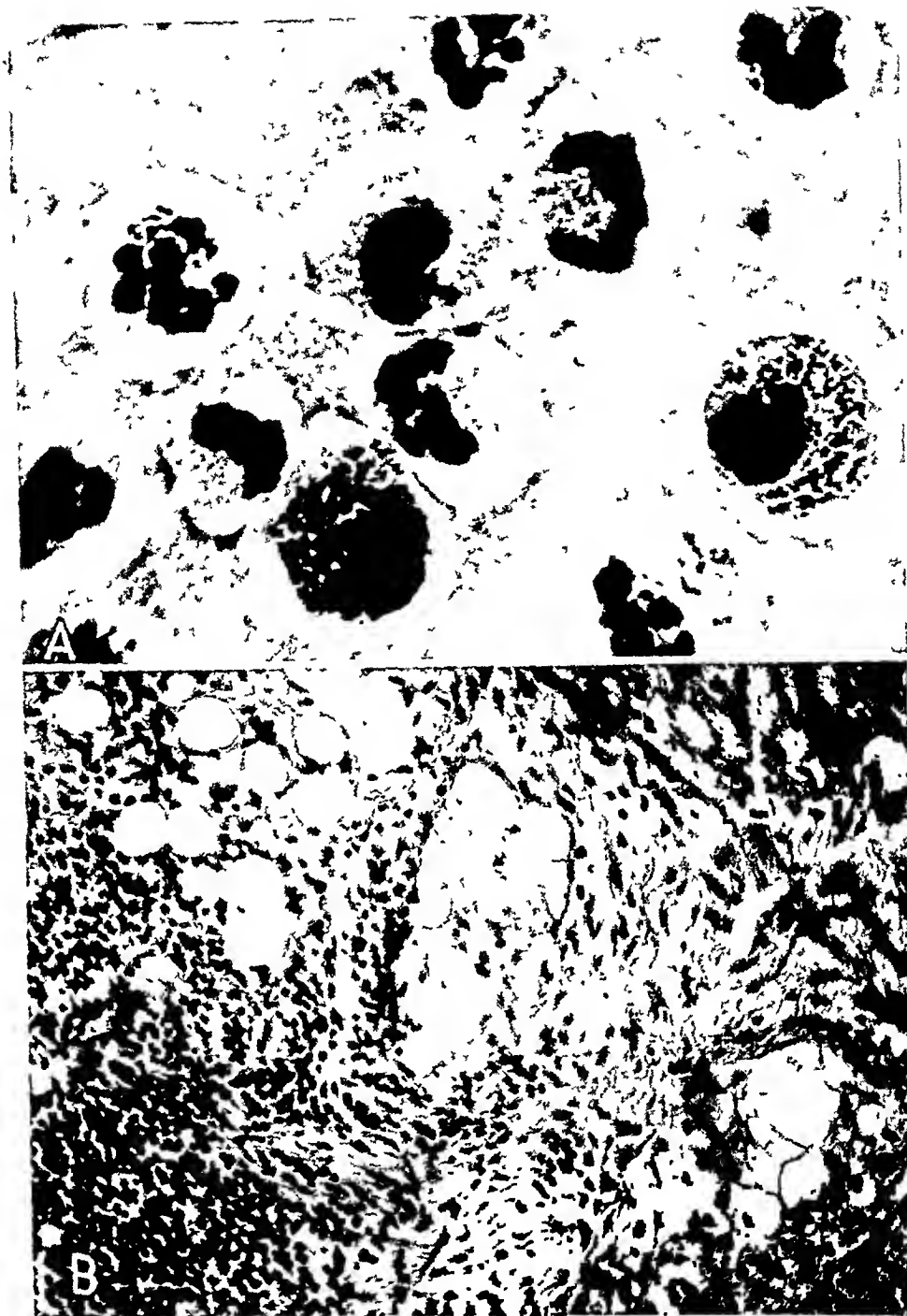


Fig. 4.—*A*, oil immersion view of a film of pericardial fibrinopurulent exudate from a rabbit after a twenty-four hour course of treatment with acetylated 4,4'-diaminodiphenylsulfone hydrochloride started twelve hours after production of the pericarditis. Note the paucity of extracellular organisms and the phagocytosis of bacteria by neutrophils and macrophages, especially the latter. Gram stain;  $\times 1,600$ .

*B*, photomicrograph of a section of the heart of a rabbit after a seven day course of treatment with acetylated 4,4'-diaminodiphenylsulfone hydrochloride started twenty-four hours after the production of pericarditis. Observe the heavy infiltration of mononuclear cells associated with the deposition of fibrous tissue about remains of adipose tissue of the pericardium. Gram-Weigert staining for bacteria in sections from corresponding blocks reveals absence of bacteria. Hematoxylin and erythrosin;  $\times 260$ .

nuclear cells and the disappearance of the organisms. At this stage the neutrophils play the principal role in the disposal of the micro-organisms. The cellular exudate in the twelve hour group thereafter undergoes rapid degeneration and necrosis, disappearing completely by the fourth day after the commencement of treatment. On the other hand, the twenty-four hour group shows a progressive increase in number and phagocytic activity of the macrophages at the forty-eight hour stage and at four days. These cells become the dominating ones in the exudate at the forty-eight hour stage and are chiefly responsible for the phagocytosis of any surviving organisms. It is clear that phagocytosis plays a conspicuous and significant part in the disposal of the micro-organisms and, therefore, in the process of recovery.

Edema and fibrin are not abundant in the pericardial exudate, and necrosis of the superficial muscle fibers of the myocardium is not conspicuous. Associated with the appearance and increase in number of the mononuclear cells, there is progressive dissolution of the fibrin. At first focally distributed in the areolar tissue beneath the epicardial serosa, the mononuclear cells increase progressively in numbers, spreading diffusely over the visceral pericardium and down the fibrous septums into the superficial layers of the myocardium.

Microscopic evidences of pleuritis or of pulmonary inflammation are not observed at any stage in the sulfone-treated groups. Histologically, the kidneys reveal no deposit of crystalline material in the renal tubules and pelvic cavities nor was there evidence of "pyelonephritis" possibly resulting from irritation by crystals of the drug.

Healing in the twenty-four hour interval animals treated with the sulfone compound takes place chiefly by organization with granulation tissue. On the fourth day after the initiation of treatment the reparative phase of the healing process, marked by abundant proliferation of young fibroblasts and capillaries, overlaps the exudation of mononuclear cells (fig. 4 B). Accompanying the disappearance of the mononuclears there is progressive deposition of fibrous tissue, ultimately leading to fibrous union of the two pericardial layers and bacterial sterilization of the sac.

#### RELATIONSHIP BETWEEN WHITE BLOOD CELL COUNT, BACTEREMIA AND MORTALITY

Early and initially in the control animals there is leukopenia due very largely to the reduction in number of the neutrophils. This leukopenia persists until death, becoming maximum at twenty-four to ninety-six hours after infection, with the number of white cells ranging between 3,000 and 4,500 per cubic millimeter.

In the twelve hour group of rabbits treated with the sulfone compound leukocytosis (leukocyte counts from 15,000 to 30,000) is nearly

always present at twenty-four to forty-eight hours after the institution of treatment. At times rising to higher levels, it is maintained for another two to five days, the number of white cells declining rapidly to normal thereafter.

In the twenty-four hour sulfone-treated group the initial leukopenia generally continues for another day or so after the onset of the pericarditis. The total white cell count then rapidly rises to 20,000 or more in the large majority of recovered animals. On the other hand, most of the animals succumbing to the infection show a progressive reduction in the white cell count to levels of 4,500 or lower after temporary leukocytosis.

The thirty-six hour treated group reveals early profound depression of the white cell count below normal. Except for infrequent brief rises to normal or above, the depressed white cell count persists in 86 per cent of the fatal infections.

The number of circulating leukocytes and the fluctuation thereof constitute a significant prognostic but by no means infallible measure of the probable course of the infection and of its response to treatment. The white cell counts at the twenty-four hour and forty-eight hour stages of infection prove to be more significant indicators of the outcome of the suppurative pericarditis at this time than the bacteremia. The fatality rate in all the sulfone-treated animals with counts of 20,000 or more after one to two days of treatment in the groups whose treatment was started after twenty-four hours is low.

There is a striking relationship between the time of appearance and the degree of bacteremia and the level of the white blood cells. The number of leukocytes is roughly inversely proportional to the frequency of bacteremia, which occurs least often with persistent white cell counts of more than 20,000. In general, the earlier and the more profound the depression of the white cell count, the higher is the incidence of bacteremia and the higher the colony count of streptococci per cubic centimeter of blood. Thus, in groups C and D, rabbits showing persistent counts of 4,000 or less, the blood cultures showed 1,000 or more colonies of streptococci per cubic centimeter of blood, with a resultant mortality of nearly 100 per cent. With disappearance of the organisms from the circulating blood as the result of treatment, the white cell count rapidly rises twenty-four to forty-eight hours later to normal limits or higher. The development of continued and progressive bacteremia in the face of treatment is indicative of impending death and provides more significant information as to the outcome of the infection at the forty-eight hour and seventy-two hour stage and thereafter than the total white cell count.

A distinct correlation exists between the time of onset and the severity of the bacteremia and the death rate. Thus, the earlier and

the more severe the bacteremia, the higher is the fatality rate. Progressive bacteremia with 1,000 colonies or more per cubic centimeter of blood, resistant to treatment, invariably proves fatal. In the control group the organisms may be detected in the circulating blood as early as twelve hours and always at forty-eight hours after the inception of the infection. The aggregation of streptococci at the twelve hour stage averages from 50 to 200 per cubic centimeter of blood. The number of circulating organisms increases in a strictly geometric progression, so that shortly before death they number 100,000 per cubic centimeter of blood or more. This number appears to constitute the lethal level.

#### SUMMARY

It has been shown for the strain of beta hemolytic streptococcus investigated that an acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride exercises a distinctly beneficial effect on the natural course of experimental beta hemolytic streptococcus pericarditis in rabbits. It considerably increases the number of cured survivors in comparison with their complete absence in the sulfanilamide-treated group, and definitely prolongs the average duration of life beyond that of the control and that of the sulfanilamide-treated group. The most effective results were obtained in a group of 50 rabbits whose treatment was initiated twelve hours after the production of the pericarditis; 49 of these animals were cured. The incidence of survivors and the prolongation of life are definitely related to the time of starting treatment.

The toxicity of this compound is of a relatively low order.

The results are on a scale sufficiently large to indicate that the acetylated derivative of 4,4'-diaminodiphenylsulfone hydrochloride is far superior to sulfanilamide in its therapeutic effect on beta hemolytic streptococcus infection of the pericardium as observed in rabbits.

# STUDIES OF CARTILAGE

## IV. A MORPHOLOGIC AND CHEMICAL ANALYSIS OF AGING HUMAN COSTAL CARTILAGE

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There is a belief that at least three systems govern the deposition of calcium in human tissues. One is concerned with the regulation of special ionic equilibriums in circulating bodily fluids. The second is concerned with particular activities of cells in those regions which undergo calcification. The third is the matrix in which calcium is deposited. No theory of calcification comprehends the means by which the three systems operate. The importance of the regulation of special ionic concentrations in the blood has been established, but the basic determinants of calcification lie within the sphere of composition and function of specialized cells and matrices. One of the simplest examples of the participation of the second and third systems is the gradual deposition of calcium in aging costal cartilage. In this report the sequences in this process are described histologically and by quantitative analyses for lipids, iron, calcium and chondroitin-sulfuric acid.

### METHODS

*Preparation of Cartilage.*—Tissue was obtained post mortem from 21 persons, aged 2 to 73 years. The third to sixth costal cartilages were resected and the perichondrium scraped away from the underlying matrix. The cartilages, free from perichondrium and other adherent tissues, were then divided transversely into blocks measuring 8 mm. in length. Several blocks of uniform appearance were selected from the group. If calcium deposits were recognized, only those blocks with similar axial deposits were retained for analysis. All blocks containing massive deposits of calcium and bone, especially in subperichondrial areas, were discarded.

The selected blocks, usually 3 to 5, were divided transversely into two parts, one part measuring about 3 mm. in length and the other about 5 mm. The smaller segments were prepared in permanent sections for microscopic study. The larger blocks, while still fresh, were cut with a freezing microtome into sections averaging 20 to 25 microns in thickness. As a rule, no difficulty was encountered in cutting the sections, for any calcified tissue that could be cut in the fresh state with a knife could be cut in the frozen state with a microtome. In handling the sections, however, densely calcified areas of the matrix often broke away. These remnants were discarded.

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*Determination of Calcium in Sections of Cartilage.*—The fresh frozen sections, weighing 300 to 600 mg., were washed in distilled water and then transferred to a volume of two-tenths molar acetate buffer solution,  $p_H$  4.8, approximating 1 cc. for each 2 mg. of tissue. After decalcification at 5 C. for twenty-four hours, the sections were filtered from the buffer solution over a sintered glass disk, washed with distilled water, dried in vacuo and then dried to constant weight at 110 C. In this way the value for the quantity of decalcified cartilage was obtained.

In determining the quantity of calcium extracted from the cartilage, the calcium was precipitated from the buffer solution as calcium oxalate and the estimation completed by alkalimetric titration of the oxide.<sup>1</sup>

The values obtained for calcium by this method were in good agreement with those from control analyses of the ashes of selected samples.

*Determination of Lipids.*—Each dry decalcified lot of sections was extracted for twenty-four hours under a reflux condenser with a boiling solution containing equal parts of ethyl ether and petroleum benzine. The extract was reduced to dryness and the weight of the ether-soluble residue obtained.

*Hydrolysis of Cartilage After Extraction of Calcium and Lipids.*—After extraction of calcium and lipids, 125 mg. of cartilage was resected from each dry lot of sections. There were 24 samples, 21 of which were from different cases. Six samples were obtained from different pairs of costal cartilages in 3 cases. Each sample was placed in a long pyrex tube containing 9.5 cc. of 4.2 normal hydrochloric acid and 0.5 cc. of 10 per cent barium chloride. The tubes were sealed in a flame and the contents hydrolyzed for seven hours at 100 C.

*Determination of Sulfate Liberated by Hydrolysis.*—During hydrolysis, sulfate ions precipitated as barium sulfate. After completion of the period of hydrolysis, the sealed tubes were broken and an additional 0.25 cc. of 10 per cent barium chloride added to insure near quantitative precipitation. The hydrolysates were cooled and filtered. The residues were washed and ashed. The ash of each sample was weighed and the sulfate computed.

*Determination of Reducing Substances in Hydrolysates.*—One cubic centimeter of each hydrolysate was used for quantitative determination of reducing substances by the micromethod of Folin, modified as required by conditions.<sup>2</sup>

*Determination of Iron in Hydrolysates.*—Each hydrolysate, minus 1 cc. removed for analysis of reducing substances, was dried. The residues were ashed with nitric acid and a 30 per cent solution of hydrogen peroxide. Iron content was determined by the method of Lintzel.<sup>3</sup>

*Estimation of Chondroitin-Sulfuric Acid.*—The quantity of chondroitin-sulfuric acid in small amounts of cartilage can be estimated without great error if the quantities of reducing substances and sulfate in hydrolysates of the cartilage are known.<sup>2</sup> Approximately one-half the total quantity of reducing substances is derived from chondroitin-sulfuric acid. Essentially all sulfate recovered from hydrolysates of cartilage, previously extracted at  $p_H$  5 to remove the labile non-diffusible fraction, is a degradation product of chondroitin-sulfuric acid. As it was advisable in the present study to work with a small amount of tissue, the

1. Fiske, C. H., and Adams, E. T.: J. Am. Chem. Soc. **53**:2498, 1931.
2. Hass, G. M., and Garthwaite, B.: Arch. Path. **33**:145, 1942.
3. Lintzel, W.: Ztschr. f. d. ges. exper. Med. **86**:269, 1933.

quantities of polysaccharide were routinely computed from data obtained by analyses of reducing substances and sulfate.

Because of the possible error in the indirect method of determining the quantity of polysaccharide, the conclusions from the microchemical data were checked by macrochemical semiquantitative isolation of the polysaccharide from tissues of selected cases. For this purpose, surplus cartilage from the patients in cases 8, 13, 18 and 22, aged 35, 47, 60 and 66 years respectively, was ground into fragments, dried by alcohol-ether extraction and then pulverized. The polysaccharide was isolated as the barium salt from 5 Gm. of each sample by the method of Levene.<sup>4</sup> One gram of each sample was ashed with nitric acid and a 30 per cent solution of hydrogen peroxide. Calcium was recovered from the ash as calcium oxalate and the quantity calculated from the weight of the ignited precipitate.

*Morphologic Study.*—A representative part of each block of cartilage used for chemical analysis was available for gross and microscopic study. In the gross study, records were made of the resilience, fragility, fibrillation, pigmentation and calcium deposits. The blocks were then fixed in 4 per cent solution of formaldehyde U. S. P., decalcified and embedded in paraffin. Permanent sections were stained with hematoxylin and eosin for microscopic study. In this study cells were counted in several peripheral and central fields of the matrix. Morphologic changes in the structures of cells were noted. Rough estimations of the percentage of the matrix occupied by osteoid tissue, bone, bone marrow and fibrillary or other disintegrative changes were made.

#### RESULTS

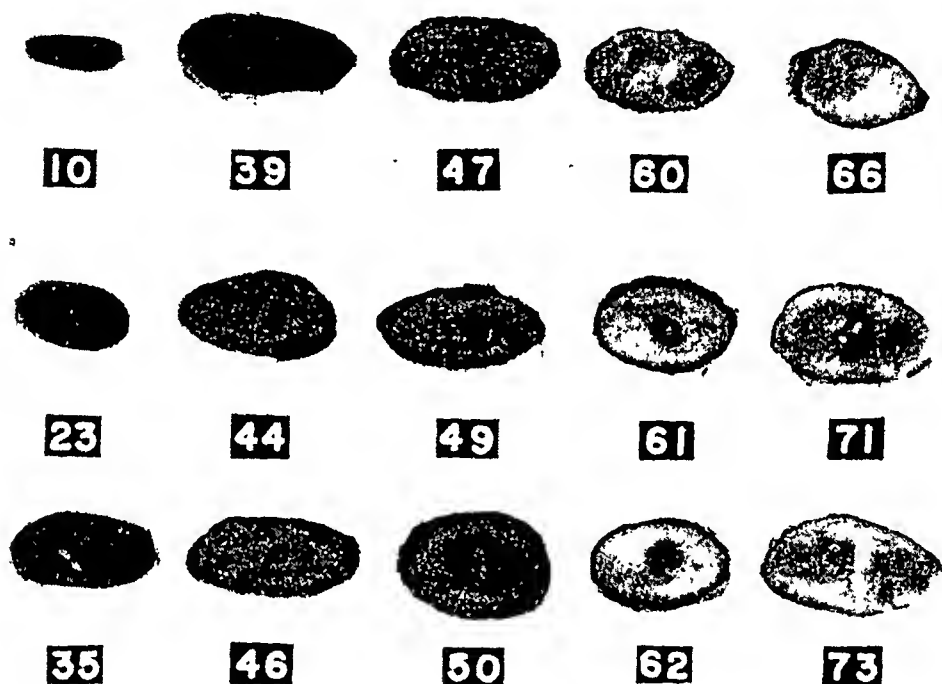
*Gross Morphologic Changes.*—From birth until middle life there is a progressive increase in the cross-sectional area of costal cartilage (figure, ages 10 to 39). During this period of growth the tissue is white, homogeneous, translucent and resilient. By the fourth decade of life several changes, especially in the axial region of the cartilage, begin to appear. There is a pale yellow pigmentation (figure, ages 35 to 44 years). The tissue is more opaque. Patches of linear fibrillary striations are found (figure, ages 44, 46 and 47). The resiliency is decreased. As these changes progress through the fourth and fifth decades, deposits of calcium usually become recognizable. The deposits are more diffuse than circumscribed and usually appear first in the pigmented central areas (figure, age 49). With increasing age the average amount of calcium increases, with partial restoration of white color to the tissue (figure, ages 49 and 50). Islands of bone and bone marrow appear, especially in densely calcified areas (figure, ages 61 and 62). Fibrillation and brown pigmentation, the former irregularly and the latter diffusely, spread from the axial area toward the perichondrium (figure, ages 35 to 62). The only cartilage which retains youthful appearance is that which lies just beneath the perichondrium (figure, ages 49 to 62). Even this at times is modified by atypical proliferation of the perichondrium, calcification and osteogenesis. Finally, in the eighth decade the central

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4. Levene, P. A. T.: Hexosamines, Their Derivatives and Mucins and Mucoids, Monograph 18, Rockefeller Institute for Medical Research, 1922.

areas of cartilage often are occupied by irregular white deposits of calcium and circumscribed islands of bone (figure, ages 71 and 73). Elsewhere, the matrix, even beneath the perichondrium, is brown, fragile and often fibrillary (figure, ages 71 and 73).

*Microscopic Morphologic Changes.*—In young costal cartilage, there is continuous proliferation of cells of the perichondrium, with differentiation of the proliferating cells into chondrocytes and production of intercellular matrix. Hence, peripherally the ratio of cells to matrix is high, and this ratio decreases as the cells become enveloped in an abundant matrix (table 2). As maturity is reached, the proliferation of cells



Gross appearance of costal cartilage at various ages.

ceases and the ratio of cells to matrix approaches a minimum late in the third and early in the fourth decade of life. In this period several microscopic changes begin to appear. Granular deposits of calcium are present in the matrix, especially in metachromatic regions. Local parallel bundles of prominent fibrils are encountered. These are first recognized in the axis of cartilage in areas which are elective sites of initial pigmentation and calcium deposition. With increasing age, fibrillation usually becomes coarse and more widespread (table 2). There is a coincidental decrease in the number of cells, and those which remain often exhibit morphologic changes of diminishing viability. This is especially prominent in many areas where the homogeneous or fibrillated matrix has undergone disintegration. In these areas the normal orderly

TABLE 1.—*Chemical Analysis of Cartilage\**

Case	Sample	Age	Lipid, per Cent	Reducing Substances, per Cent	Sulfur as SO <sub>4</sub> , per Cent	Iron, per Cent	Calcium, per Cent
10507	1	2	0.98	25.6	0.81	0.017	0.046
10450	2	10	0.07	28.6	1.00	0.021	0.062
10496	3	11	0.52	27.7	0.95	0.012	0.099
10503	4	12	0.04	27.1	0.86	0.028	0.155
10509	5	21	0.03	31.6	1.21	0.024	0.177
10524	6	23	0.11	31.6	1.46	0.004	0.278
10455	7	30	0.14	30.8	1.37	0.009	0.327
10567	8	35	0.08	30.3	1.30	0.010	0.255
10500	9	39	0.51	30.0	1.03	0.014	3.147
10462	10	39	0.12	29.7	1.37	0.015	0.257
10561	11	44	0.01	26.7	1.09	0.008	0.249
10498	12	46	0.12	26.9	1.18	0.008	0.481
10535	13	47	0.13	28.1	1.06	0.016	0.280
10388A	14	49	0.38	27.2	1.05	0.006	2.622
10388B	15	49	0.49	27.0	1.01	0.010	0.900
10389A	16	50	0.40	30.8	1.17	0.014	1.166
10389B	17	50	0.17	30.4	1.19	0.012	0.501
10385A	18	60	0.42	28.3	0.92	0.011	1.980
10385B	19	60	0.43	27.3	0.88	0.032	6.693
10400	20	61	0.45	23.6	0.85	0.021	2.414
10503	21	62	1.52	22.4	0.66	0.027	4.067
10504	22	66	0.13	24.6	0.68	0.011	0.758
10525	23	71	0.06	19.8	0.61	0.008	1.926
10529	24	73	0.11	24.3	0.80	0.024	1.186

\* All values are expressed as percentages of ether-extracted decalcified tissues.

TABLE 2.—*Comparison of the Microscopic Appearance of Cartilage with the Amounts of Chondroitin-Sulfuric Acid and Calcium*

Sam- ple	Age, Yr	Chondrocytes, Average Number per Unit Area		Percentage of Cross-Sectional Area				Percentage of Chondroitin-Sulfuric Acid* Estimated by;			Percent- age of Calcium *
		Periph- eral	Cen- tral	Osseous		Disintegrations		Reduc- ing Sub- stances	Sulfate	Chem- ical Isola- tion	
				Matrix	Mar- row	Fibril- lary	Other Types				
1	2	45	29	0	0	0	0	14.1	12.1	....	0.046
2	10	52	20	0	0	0	0	18.1	15.0	....	0.062
3	11	46	14	0	0	2	1	16.9	14.2	....	0.099
4	12	41	17	0	0	2	0	16.1	12.9	....	0.155
5	21	26	12	0	0	5	0	22.1	18.2	....	0.177
6	23	28	12	0	0	10	2	22.1	21.9	....	0.278
7	30	24	14	0	0	5	2	21.1	20.6	....	0.327
8	35	23	9	0	0	10	5	20.4	19.5	11.4	0.255
9	39	27	14	5	5	5	1	20.0	15.5	....	3.147
10	39	20	15	0	0	5	5	19.6	20.6	....	0.257
11	44	22	9	0	0	5	2	15.6	16.4	....	0.249
12	46	20	8	0	0	20	1	15.9	17.7	....	0.481
13	47	27	14	0	0	10	0	17.5	15.9	8.5	0.280
14	49	32	11	5	2	2	5	16.3	15.8	....	2.622
15	49	21	14	0	0	5	5	16.0	15.2	....	0.960
16	50	16	10	0	0	5	0	21.1	17.6	....	1.166
17	50	21	13	0	0	10	2	20.5	17.9	....	0.501
18	60	22	10	5	2	5	2	17.7	13.8	6.9	1.980
19	60	24	12	10	10	10	5	16.4	13.2	....	6.693
20	61	14	7	2	1	10	2	11.5	12.8	....	2.414
21	62	25	9	2	2	15	5	9.9	9.9	....	4.067
22	66	27	11	2	2	20	10	12.8	10.2	4.8	0.758
23	71	13	10	0	0	25	10	6.4	9.2	....	1.926
24	73	18	8	2	2	15	15	12.4	12.0	....	1.186

\* The values are calculated as percentages of ether-extracted decalcified tissues!

structure is replaced by granular, globular and nondescript discontinuous acellular clumps of abnormal matrix (table 2). But in many fibrillary, densely calcified fields the cells often are well preserved. It is in these areas that osteoid tissue, bone and fatty bone marrow, more or less occupied by hemopoietic foci, appear. These new tissues develop, especially in axial densely calcified areas, but the organization is unlike that encountered in normal enchondral ossification centers, and they appear long after the time of closure of epiphysial lines (table 2).

*Quantity of Lipids.*—The quantities of ether-soluble substances are recorded in table 1. As indicated by the microscopic studies, these were extracted not only from cartilage but also from adventitious intracartilaginous islands of bone and bone marrow. The values range from 0.01 to 1.52 per cent. In comparison with the values from most human tissues these values are very low. Several high values are to be attributed to islands of fat cells in marrow spaces. Variations in values are also partly due to unavoidable contamination of cartilage with extracartilaginous adipose tissue during removal of the perichondrium. Hence, the low values in each age group are the more correct ones. The uniformity of these values indicates that the lipid content is independent of any of the observed changes in aging cartilage. Furthermore, it is improbable, in view of the constant low figures, that extracellular accumulations of lipids, which are often associated with calcification of other intercellular matrices, have any bearing on calcium deposition in cartilage.

*Quantity of Iron.*—The quantities of iron recovered from cartilage after removal of lipids and calcium are recorded in table 1. The values are low and are independent of age or changes in the matrix incidental to calcium deposition. It is probable that higher values would have been obtained by a study of the ash prior to decalcification. The present values show that if iron is implicated as a precursor of calcium in cartilage it is not firmly bound to cells or matrix.

*Quantity of Pigment.*—The supposition that pigmentation of the matrix is due to the presence of lipids or iron was not confirmed by analysis. The pigment was not extracted with ether or with a buffer solution,  $p_H$  4.8. Neither was there any relationship between the intensity or the quantity of pigmentation and the quantity of iron. Nor was the pigment detected microscopically as one of the "wear and tear" pigments often found in the cytoplasm of cells of old people.

The quantity of pigment in cartilage increases with age. It first appears in axial parts of cartilage that are destined to undergo calcification and in time gradually spreads throughout the tissue. Speculation indicates that it may be a degradation product of chondroitin-sulfuric acid, for it appears as the polysaccharide decreases in quantity. The

possible role of the pigment in the promotion of calcification deserves further consideration.

*Quantity of Calcium and Bone.*—The data in table 2 show that the quantity of calcium increases with age. This increase begins in the first decade and progresses, more rapidly in some than in others, to reach high values in all cartilage over 50 years of age. When high values were encountered in young cartilage, there was ossification (table 2). This was also the rule when the quantity in aged tissue was unusually great. In general when the content of calcium at any age was greater than 1 per cent, there was ossification. This rule could not be strictly applied, because of variations in the volume of matrix occupied by a given amount of calcium, but it confirmed the impression that ossification did not begin until a large quantity of calcium had been concentrated in the area. In other words, in the 4 samples of cartilage with the highest calcium values, the amount of bone, even if fully calcified, would account for only a minor fraction of the calcium.

*Quantity of Chondroitin-Sulfuric Acid.*—The microchemical and random macrochemical estimations of the quantities of chondroitin-sulfuric acid were in good agreement though, as expected, the yields of purified polysaccharide were much lower than the predicted yields (table 2). This may be attributed to inevitable losses incidental to isolation and purification of the product.

The independent estimations of the content of polysaccharide by use of data referable to reducing substances and sulfate were in fair agreement (table 2). In accordance with conclusions from previous studies, about one half of the maximum quantity of reducing substances was derived from sources other than chondroitin-sulfuric acid.<sup>2</sup> Hence, about 15 per cent must be subtracted from each experimental value in order to obtain the percentage derived from the polysaccharide. The theoretic yield of reducing substances from chondroitin-sulfuric acid is about 75 per cent by weight in terms of dextrose. Data obtained by use of this figure and corrected values for reducing substances show that the amounts of chondroitin-sulfuric acid range from 6.6 to 22.1 per cent. A corresponding calculation with values for sulfur recovered as sulfate indicates that the amounts of the polysaccharide vary from 9.2 to 21.9 per cent. The amounts recovered by semiquantitative isolation varied from 4.8 to 11.4 per cent. All estimates are recorded in table 2.

In general, the quantity is low in infancy. It progressively increases to a maximum in the third and fourth decades of life. Thereafter, it decreases so that in the late seventh and eighth decades the content is less than half maximum values. With this decrease there are numerous simultaneous changes in the cartilage. Among these changes, a progressive increase in the amount of calcium is encountered, though no

quantitative relation seems to exist between the depletion of polysaccharide and the deposition of calcium. Nor is there any established quantitative relation between the depletion of polysaccharide and any gross or microscopic change. If any does exist, a revision and refinement of method will be required before it can be clearly exposed. This analysis is not beyond the scope of available histochemical micro-methods.<sup>2</sup>

#### COMMENT

Physicochemical studies have shown that the deposition and the resorption of the calcium salts are regulated within limits by changes in special ionic concentrations in circulating bodily fluids. But this is a restricted regulation and fails to account for the fact that calcium salts are deposited neither diffusely nor indiscriminately but in elective sites. Among these elective sites, the matrix of cartilage is perhaps the best example, and it is the object of the present study. Costal rather than epiphyseal cartilage was chosen for study because in it calcium is deposited in a simple tissue with minimal complication by formation of osteoid tissue and bone. Morphologic studies were planned so that an idea was obtained of the structure of each sample of cartilage subjected to chemical analysis. Analyses for iron and lipid were made to test the validity of theories which implicate these materials in the mechanism of calcification and to inquire into the possible relation of these materials to the brown pigmentation of aged cartilage. The estimation of the content of chondroitin-sulfuric acid was made because the large amount of this compound in normal uncalcified cartilage is the chemical characteristic by which cartilaginous matrices are best distinguished from avascular intercellular matrices, which do not ordinarily calcify.

The lipid content throughout life is constantly at such a low level that the conclusion is reached that lipids bear no quantitative relation to any observed change in the morphologic appearance or the chemical composition of cartilage.

The iron content of cartilage after extraction at  $p_H$  4.8 is very low and relatively constant throughout life. The data justify no conclusions with respect to the participation of iron in calcification or in pigmentation of the matrix.

The amount of chondroitin-sulfuric acid is variable. The low value in early life is to be attributed to the low ratio of matrix to cells. As this ratio increases, the amount of polysaccharide increases up to a maximum in the third or the fourth decade of life. At this age, despite further increase in the ratio, the quantity of polysaccharide begins to decrease, calcium deposition is accelerated in the axes of the cartilages and morphologic changes begin to develop. Generation of chondrocytes by the perichondrium is inconspicuous. Signs of diminished viability are detectable in cells located deep in the matrix. Pale yellow pigmen-

tation and occasional foci of fibrillation appear in central axial areas. With increasing age all changes are accentuated. The quantity of polysaccharide falls to less than half maximum values. Calcium deposits increase in density, especially centrally, spreading outward in diminishing concentration toward the perichondrium. Small foci of osteogenesis appear, particularly in the dense areas of calcification. Generation of chondrocytes ceases. Cells everywhere, though they show remarkable persistence, display the usual morphologic changes of diminished viability. The light yellow pigmentation changes to brown and spreads throughout the tissue except in densely calcified areas. Areas of fibrillation are often prominent. The tissue is firm and brittle. This is the usual status in the eighth decade of life.

These data constitute an incomplete histochemical description of calcification rather than an explanation of the process. The description reveals morphologic and chemical coincidences in sequence but fails to specify causes and effects. However, the following speculations are in accord with the observations. So long as the polysaccharide content of cartilage remains at a high level, the amount of calcium in the matrix remains at a low level, although calcium is acquired in limited quantities during the period in which there is an apparent increment of polysaccharide. As the quantity of polysaccharide decreases with increasing age, there is a comparable, though variable, increase in calcium in the matrix. But this is not the only accompaniment of disappearance of polysaccharide. The number of cells in the matrix diminishes, and those which remain display morphologic changes which are ordinarily accepted as evidence of diminished activity, if not of decreased viability. But the functional capacity of those cells which persist in considerable numbers may still be very important from the standpoint of calcification. There is little doubt that one function of the cells is synthesis of polysaccharide or its precursors, and it is reasonable to assume that the limitation of this function in aged cartilage is responsible for the low polysaccharide values. In other words, the preservation of matrix in a youthful state is contingent on the continuous replacement of polysaccharide, and if this does not occur, the quantity of polysaccharide decreases. With the decrease, the increased rigidity, friability, fibrillation and pigmentation of the tissue become evident. Indeed, it is probable that fibrillation of the matrix is simply an exposure of fused elementary fibrils by disappearance of interfibrillar polysaccharide. A similar argument applies to the cause of loss of resilience of the matrix. The composition, source and significance of the pigment are unknown. It increases as polysaccharide decreases and appears first in the axial zones which are elective sites of calcification. It may be a degradation product of polysaccharide and unrelated to the mechanism of calcification, but



because of the spatial and temporal coincidence of pigmentation and calcification the pigment deserves further study.

Finally, the conclusion is reached that the mechanism by which the intercellular matrix is maintained in a healthy state is the mechanism which fails and in failing leads to a succession of complex changes. The concrete chemical evidence of this failure is not calcification primarily but is depletion of chondroitin-sulfuric acid. Indeed, one oversimplified interpretation of the data is that maintenance of a high level of chondroitin-sulfuric acid is a device by which cartilage is protected against calcification.

#### SUMMARY

From infancy until the fourth decade of life the amount of chondroitin-sulfuric acid in costal cartilage increases in proportion to the increase in the ratio of matrix to cell. In later life, despite further increase in the ratio, the amount of polysaccharide decreases. Coincidental with this decrease, several changes appear, especially in the central areas of the cartilage. Calcium is deposited. A diffuse yellow pigment is detectable. Foci of fibrillation develop. The rate of differentiation of chondrocytes from the perichondrium decreases, and cells located deep in the matrix show early signs of diminished viability. With increasing age these changes become more conspicuous, so that in the eighth decade the amount of chondroitin-sulfuric acid has been reduced to less than half the maximum value. Calcium deposits are prominent. Islands of tissue disintegration, osteoid matrix and bone are often present, especially in densely calcified fields of the cartilaginous matrix. The yellow pigment, which is neither lipid nor iron, now contributes a brown tinge to all cartilage except the white densely calcified areas. The foci of fibrillation, initially central in location, are more numerous and widely distributed. There is no proliferation of chondrocytes from the perichondrium, but most mature cells throughout the tissue persist despite long-continued signs of diminished viability.

The spatial and temporal relationships among these progressive changes indicate that calcification of cartilage in the presence of an appropriate supply of necessary ions depends on failure of the mechanisms responsible for maintenance of the intercellular matrix.

## Case Reports

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### MYCOTIC AND DISSECTING ANEURYSMS OF THE AORTA COMPLICATING BACTERIAL ENDOCARDITIS

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A dissecting aneurysm resulting from a mycotic aneurysm in the case reported here represents a rare complication of bacterial endocarditis. Because of the unusual combination of lesions it seems pertinent to put this case on record.

#### REPORT OF A CASE

W. R., a 75 year old man, was admitted to the Boston City Hospital on Jan. 22, 1940, because of inability to void urine since a few days before. He was irrational and unable to give a lucid history.

When examined, he was thrashing about in bed. The lungs were normal. The heart was enlarged to percussion, and a loud systolic murmur was heard over the precordium. No diastolic murmur was heard. The prostate was generally enlarged and firm. The penis was absent, the urethral opening being level with the abdominal wall. There was slight pitting edema of the legs. The blood pressure was 122 systolic and 58 diastolic.

The hemoglobin content was 88 per cent, and the total leukocyte count was 21,800 per cubic millimeter. The urinary sediment showed 20 to 25 white blood cells per high power field.

The patient remained irrational and incontinent. Rales developed at the bases of both lungs with a concomitant rise in temperature. Death occurred on the sixth hospital day.

The final clinical diagnoses were: arteriosclerotic heart disease with mild decompensation; pyelonephritis; operative absence of the penis; benign prostatic hypertrophy, and intertrigo.

*Postmortem Examination.*—Externally the body was that of a well developed and well nourished elderly white man. There was an umbilical hernia, 2.5 cm. in diameter and 0.8 cm. in height. The skin over the legs was purple and scaly. The penis was absent, having been amputated at the level of the penoscrotal junction. The peritoneal cavity was without pathologic change, and each pleural cavity contained about 400 cc. of clear straw-colored free fluid.

The pericardium contained about 300 cc. of partly clotted blood. There was no demonstrable perforation in the heart or in the large vessels associated with

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From the Mallory Institute of Pathology of the Boston City Hospital.

the pericardial cavity. The blood was presumably derived by slow leakage from the adventitia of the ascending aorta, which showed the bluish purple mottling characteristic of hemorrhage. The heart was somewhat enlarged, weighing 520 Gm.

The aortic valve was the site of greatest interest (fig. 1), exhibiting the characteristics of acute bacterial endocarditis involving adjacent portions of the left posterior and anterior cusps. The left posterior cusp exhibited a friable papillary gray and brown vegetation, measuring 1.5 cm. by 0.5 cm., well attached



Fig. 1.—Drawing of heart and aorta. The heart and aorta have been opened, exposing the left ventricular chamber and the aortic valve. Two cusps of the aortic valve show the vegetations of acute bacterial endocarditis with ulceration of the commissure between these two cusps. A dissecting aneurysm is present in the aorta. In the insert the anterior aortic leaflet has been reflected, exposing an ulcerated lateral wall of the corresponding sinus of Valsalva. At the upper limit a mycotic aneurysm is present. This is continuous with the dissecting aneurysm as shown by the arrow.

to the sinus surface of the anterior one quarter of the cusp. Beneath this the leaflet was ulcerated through to the contact surface. The anterior leaflet showed a process similar in kind but more extensive, so that papillary vegetations projected from the sinus as well as from the contact surfaces, and fully half of

the cusp was involved. There were several points of ulceration through the cusp associated with complete detachment of the cusp from its left commissural attachment. The ulcerative process extended to the commissure which lay between the two cusps described. In addition the wall of the anterior sinus of Valsalva showed considerable ulceration; no recognizable aortic tissue was present, and there was a moderate degree of aneurysmal dilatation of this sinus. This ulcerative process extended upward on the aortic wall opposite the anterior leaflet to a level just above that of the free margins of the cusps. The uppermost limit of the aortic lesion was demarcated by a row of papillary friable vegetations on the aortic wall. Just beneath these in the aorta there was an ovoid defect, measuring 1.2 cm. by 0.5 cm., through which the lumen of the anterior sinus of Valsalva communicated with a typical intramedial dissecting aortic aneurysm. The defect lay between the level of the left attachment of the anterior leaflet and the origin of the right coronary artery. The dissecting

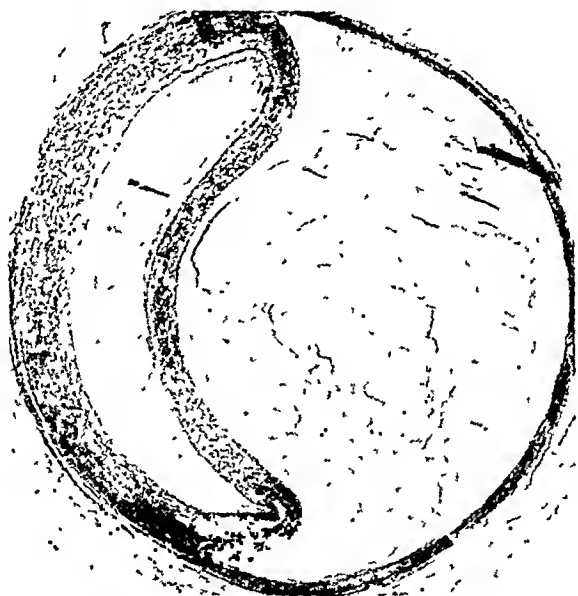


Fig. 2.—Left subclavian artery. The aortic dissecting aneurysm has extended into the left subclavian artery. The intramedial hematoma is responsible for considerable narrowing and for the crescent shape of the original lumen of the vessel.

aneurysm arose from the base of the aforementioned defect to involve the entire length of the aorta and extended into each common iliac artery. The dissection continued from here to involve from 1 to 3 cm. of the external and internal iliac arteries without rerupturing into the lumens of these vessels. The dissection also extended into the great vessels arising from the aortic arch. It rose to the level of the bifurcation in the innominate artery, for a distance of 3 cm. in the left common carotid artery and 4 cm. in the left subclavian artery (fig. 2).

The intima of the entire aorta above the root showed only a mild degree of atherosclerosis and no point of rupture.

In addition to the formation of the mycotic aneurysm at the level of the endocarditis, there was extension of the inflammatory process through the sub-

stance of the heart, the lesion appearing on the medial wall of the right auricle as a bulging reddish brown area, 1.8 cm. in diameter.

The anterior leaflet of the mitral valve showed at its base a mild degree of yellow thickening characteristic of arteriosclerosis. Otherwise the valves showed no definite evidence of any disease existing prior to the bacterial endocarditis.

The kidneys were of usual size, the combined weight being 370 Gm. The capsules stripped with ease to reveal numerous flat-based depressions typical of healed pyelonephritis and averaging 1.5 cm. in diameter. The cortical tissue between these depressions averaged 0.8 cm. in thickness and was not remarkable. There were no apparent renal hemorrhages or infarcts.

The spleen was slightly enlarged, weighing 280 Gm., and exhibited the soft, semisolid consistency frequently seen in sepsis. There were no infarcts.

The right adrenal showed two hematomas, one measuring 3.7 cm., the other 0.9 cm. in diameter. The lungs showed a moderate degree of congestion and edema. The liver was moderately enlarged and showed the yellow color characteristic of fatty metamorphosis. There was benign prostatic hypertrophy. Examination of the gastrointestinal tract revealed several small diverticula, measuring 0.7 cm. in diameter, in the sigmoid. The brain, the pancreas and the vertebral marrow were normal on gross examination.

*Microscopic Examination.*—The anterior sinus of Valsalva showed beneath the surface of its lateral wall considerable ulceration with necrosis, cellular infiltration and abscess formation (fig. 3). On the surface were deposited large collections of fibrin heavily infiltrated with polymorphonuclear leukocytes. At the upper level of this sinus the aorta was involved by an acute ulcerative process extending through the intima deeply into the wall, giving rise to a separation of the media into two layers of unequal thickness. The thicker was attached to the intima while the thinner, representing about one sixth of the medial thickness, was attached to the adventitia.

This separation represented the origin of the dissecting aneurysm described in gross. In only the lower part of the separation was there an inflammatory process. Above this the separated surfaces showed none and undoubtedly represented a tearing of the media by a stream of blood coming from the lower inflammatory area, which may be considered a mycotic aneurysm.

*Bacteriologic Examination.*—Culture of the blood from the right auricle of the heart and of the thrombus in the left common carotid artery produced no growth. *Bacillus coli communis* was obtained from the liver.

Cultures of the vegetation on the aortic valve and of the spleen and the kidney revealed pure growths of a nonmotile organism which on smear was a gram-negative pleomorphic bacillus with clubbed ends. It fermented dextrose, lactose, saccharose, xylose and mannite with the formation of acid but no gas. Even when it was transferred daily for three weeks in dextrose it did not form gas. Neither gas nor acid were formed when the organism was grown in inositol.

The reactions of the organism to certain special tests were as follows: methyl red reaction, negative; Voges-Proskauer reaction, negative; indole reaction, positive; ammonia formation, negative; catalase, positive.

*Diagnosis.*—The conditions finally diagnosed were: acute bacterial endocarditis with mycotic and dissecting aneurysms of the aorta; hemopericardium; hydrothorax; pulmonary congestion and edema; fatty infiltration of the liver; diverticulosis of the sigmoid; acute and healed pyelonephritis; benign prostatic hypertrophy; hematomas of the right adrenal, and umbilical hernia.



Fig. 3.—Lateral wall of the anterior sinus of Valsalva. In the lower portion of the photomicrograph the lateral wall of the sinus is shown ulcerated. The inflammatory process has extended through the intima into the media and is continuous with the aortic dissecting aneurysm which lies in the upper half of the illustration.

## COMMENT

Dissecting aneurysm of the aorta arising as a complication of bacterial endocarditis is a rare condition. Cases of dissecting aneurysm of the aorta may be divided into two groups: (1) those in which a primary disease of the intima is present at the site of origin of the dissection and (2) those in which the intima shows no evidence of abnormality present prior to the rupture. The second group is by far the larger<sup>1</sup> and in these cases the aneurysm is frequently associated with clinical and pathologic evidence of hypertension. In this group, postmortem examination shows no evidence of intrinsic disease of the intima but may show focal defects in the media, often referred to as idiopathic medionecrosis. Syphilitic involvement of the media is rarely seen in cases of dissecting aneurysm, and the opinion has been expressed<sup>2</sup> that the medial scars of syphilis may act as a protection against the development of a progressive hematoma of the aortic media.

Shannon<sup>1</sup> has indicated that although dissection starting at the base of an atheromatous ulcer has been mentioned as a common occurrence, this lesion occurred only seldom in the series of cases he reviewed. Thus, in a group of 218 cases of dissecting aneurysm of the aorta there were only 6 in which there was a definite statement that the dissection began in the base of an atheromatous ulcer.

Even more unusual is dissecting aneurysm of the aorta arising in the base of a mycotic aneurysm. We have been able to find only 2 cases in the recent literature. The first is the case reported by Lanbry and Bordet.<sup>3</sup> The patient, a man 40 years of age, gave a past history of rheumatic fever. During his terminal illness he was febrile, and his clinical course suggested bacterial endocarditis. Two blood cultures were negative, and one showed a tetragen. The patient died from a large hemopericardium. At autopsy the heart had vegetations on the mitral and aortic valves. The ascending aortic intima and media showed large fissures which communicated with a dissecting aneurysm lying between the media and the adventitia. The authors did not report the microscopic features and did not mention bacteriologic findings at autopsy. It is therefore difficult to accept the interpretation that the endocarditis was bacterial. From the data presented one might justifiably assume that the case of Lanbry and Bordet was one in which a dissecting aneurysm of the usual type was coincident with endocarditis.

The second case was that reported by Lippincott.<sup>4</sup> In a 59 year old man with synovitis of the knee due to infection with *Streptococcus*

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1. Shannon, T.: Dissecting Aneurysms, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

2. Weiss, S.: *New England J. Med.* **218**:512, 1938.

3. Lanbry, C. H., and Bordet, F. R.: *Bull. et mém. Soc. méd. d. hôp. de Paris* **47**:179, 1923.

4. Lippincott, S. W.: *Canad. M. A. J.* **43**:115, 1940.

haemolyticus of the beta type, septic aortitis of the lower thoracic part of the aorta developed from which a typical dissecting aneurysm arose. Bacterial endocarditis was not present in this case.

In cases of bacterial endocarditis one or more foci of acute inflammation may develop in the aortic intima with extension of the process into the deeper layers of the vessel wall and subsequent formation of a mycotic aneurysm. Usually death is due to the endocardial inflammatory process with embolic phenomena, and the mycotic aneurysm is an incidental finding at autopsy. Less commonly, the mycotic process may invade the aorta so deeply that rupture through the adventitia occurs and the patient dies of massive hemorrhage into one of the body spaces. Only rarely, as we have already indicated, does a typical progressive intramedial hematoma or a dissecting aneurysm originate at the base of a mycotic aneurysm.

The organism cultured from the valvular vegetation, the spleen and the kidney undoubtedly represents the causative organism of the endocarditis and mycotic aneurysm. We have not been able to classify this bacillus because of the extremely unusual combination of its reactions. While it is a lactose fermenter, its inability to form gas with the carbohydrates, together with its negative methyl red and Voges-Proskauer reactions, rules it out of the colon bacillus class. The lack of encapsulation, the failure to form gas, the negative methyl red and positive indole reactions and the failure to ferment inositol and to form ammonia serve to distinguish it from the Friedländer group. The organism could be distinguished from members of the dysentery group of bacilli by its prompt lactose-fermenting quality and its failure to reduce nitrates and to form ammonia. Absence of gas rules out the paratyphoid group.

For the time being the organism is given the broad classification of coliform lactose-fermenting bacillus.

#### SUMMARY

The case of a 75 year old man with acute bacterial endocarditis of the aortic valve, in whom a dissecting aneurysm of the aorta arose in the base of a complicating mycotic aneurysm is presented.

The etiologic organism was a pleomorphic gram-negative lactose-fermenting bacillus that could not be classified accurately.

The case is unusual from several angles, namely, (1) the association of bacterial endocarditis with dissecting aortic aneurysm, (2) the development of dissecting aneurysm in the base of a mycotic aneurysm and (3) the type of organism responsible for the endocarditis.



## MESOTHELIOMA (ENDOTHELIOMA) OF THE PERITONEUM

THOMAS L. RAMSEY, M.D., AND BERNHARD CHOMET, M.D., TOLEDO, OHIO

Highly characteristic conditions are produced in the peritoneum and in the pleura by primary tumors developing in these tissues.<sup>1</sup> The same is true for tumors of the pericardium.<sup>2</sup>

Considerable discussion has arisen in the literature as to whether these growths should be termed "endothelioma" or "mesoendothelioma," and recently "mesothelioma" is coming into use.

The majority of the authors are less impressed by embryologic considerations than by the morphologic and physiologic aspects of the tumor cells and by their derivation from serosal cells.

Some doubt has been expressed by several observers as to whether a true primary neoplasm ever arises from these membranes, most of the reported ones being considered as secondary carcinoma. It is not difficult to understand this contention, as these tumors do assume characteristics that cause them to resemble adenocarcinoma and some of the reported ones may have been of this nature.

We do not intend to enter into this controversial subject, and no attempt is made to review the somewhat meager and confused literature. We wish to report a case which, after thorough investigation at autopsy and careful study of many sections from involved areas, we believe to represent a true primary neoplasm arising from the serosal cells of the peritoneum.

We have adopted the name "mesothelioma," which seems best to express the origin and the nature of this neoplasm.

### REPORT OF A CASE

A white woman 62 years of age entered the hospital Oct. 2, 1940. She had been sick for about eight weeks with nausea and attacks of vomiting; she also complained of weakness and pains in the right lower quadrant of the abdomen. The pain was accentuated on standing or walking. Bearing-down sensations were present in the region of the bladder, and some pain was present during micturition.

She was an extremely obese woman. She did not appear to be in acute distress. The heart was enlarged in all diameters; the liver was also somewhat enlarged and extended about 4 cm. below the right costal margin. Slight icterus developed, and projectile vomiting with abdominal distention. The temperature became septic. The red blood cell count was 4,540,000; the hemoglobin content, 82 per cent; the white cell count was 14,000, and the differential count was polymorphonuclears 78 per cent, juvenile forms 12 per cent and lymphocytes 10 per cent. The urine was normal.

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From the Department of Pathology of St. Vincent's Hospital.

1. Ewing, J. E.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 356-359.

2. McDonald, S., Jr.: *J. Path. & Bact.* 43:137, 1936.

Roentgen examination revealed the following facts: The nonvisualized gall-bladder contained many stones. Diverticula were present in the colon. There was a small supraphrenic pouch. No defects were present in the stomach. Peristalsis progressed to the pylorus normally. The duodenal cap could not be visualized. After twenty-four hours there was no residue in the stomach; the barium sulfate was distributed throughout the colon, a small amount remaining in the terminal part of the ileum. The chest showed increased density at the left base. The cardiac shadow was definitely enlarged in all diameters; the retrocardiac space was not narrowed, however, as there was a definite increase in the dorsal kyphosis making the retrocardiac space somewhat wider than normal. The aorta appeared normal; the esophagus was normal.

The septic temperature continued; myocardial failure developed, and the patient died Nov. 20, 1940.



Fig. 1.—A portion of the involved peritoneum over the colon and a portion of the ileum with mesentery. The resemblance to plastic fibrinous peritonitis is well seen, together with large nodular areas.

*Autopsy.*—There was slight icterus, and the abdomen was somewhat distended; other external findings were not important.

The mouth, tongue and oropharynx appeared normal. The larynx, trachea and bronchi down into the smaller branches into the lungs showed no changes. The lungs were hyperemic and edematous and practically filled the pleural cavities. There was about 100 cc. of slightly turbid yellowish fluid in each pleural cavity. The pleural surfaces were smooth and glistening. There were no adhesions. There were no gross areas of consolidation in the lungs. There was 250 cc. of greenish yellow fluid in the pericardium. The heart appeared slightly smaller than normal; the musculature, valves and vessels showed no gross changes. The large vessels and other mediastinal structures, including the glands, were normal. The thymus gland was lost in the mediastinal fat.

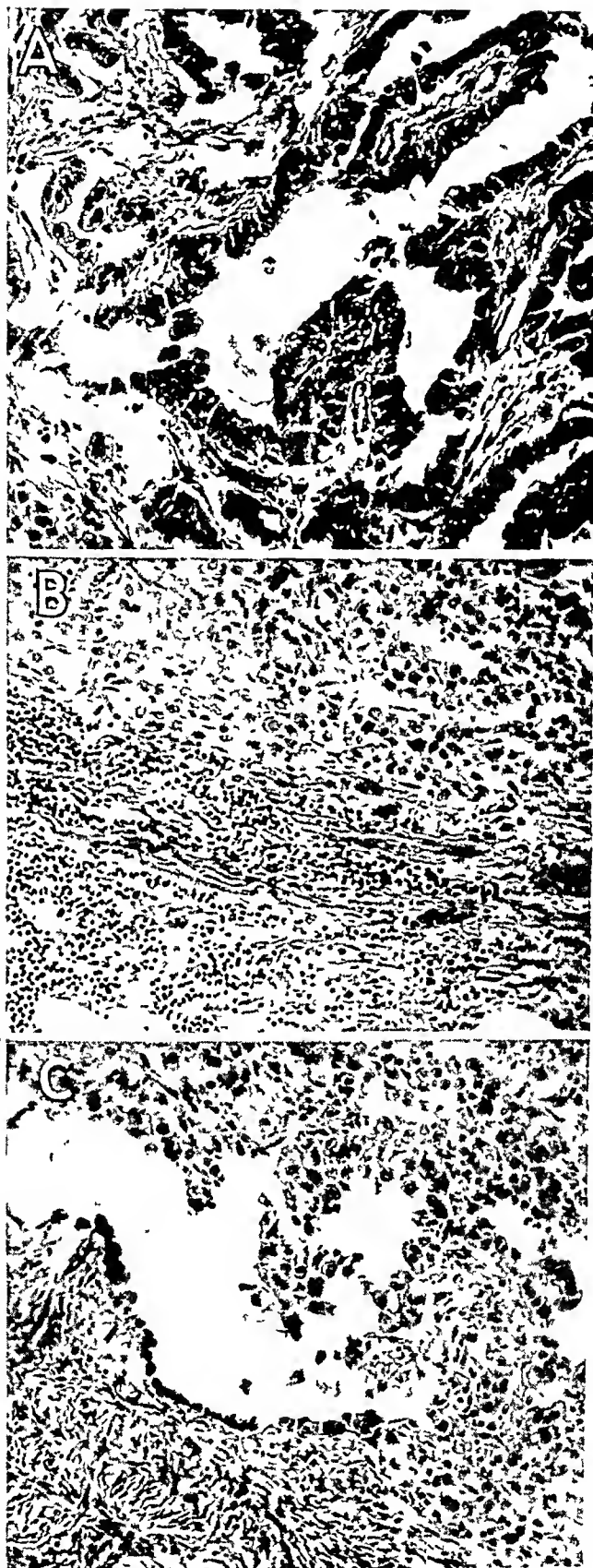


Figure 2

*(See legend on opposite page)*

There was 6 liters of slightly turbid yellowish fluid in the peritoneal cavity. The liver and spleen were high under the diaphragm. The liver was not enlarged; its substance was firm, light greenish yellow and mottled. The gallbladder was small and contracted around several faceted stones. The other biliary passages and vessels showed no gross changes.

The spleen was smaller than normal and presented chronic passive hyperemia. The pancreas was normal. The kidneys were about normal in size; the capsules were not adherent. A few small subcapsular cysts were present in each kidney. The renal pelvis, ureters and urinary bladder showed no gross changes. The adrenal glands showed autolytic changes. There was a large area of hemorrhage in the right perirenal fatty tissue extending retroperitoneally downward into the pelvic region. The renal vessels on this side showed no apparent gross lesions. The uterus, tubes and ovaries were normal except for the changes in the peritoneal coverings. One hemorrhagic cyst was present in the left ovary. The stomach, the upper part of the colon and some loops of the small intestine showed gaseous distention. The serosa over the lower parts of the intestines, including the mesentery, presented a thickened rough and nodular appearance. This was quite marked in the pelvis, extending over the fundus of the uterus and including the tubes and ovaries. Some areas presented the appearance of plastic peritonitis. Numerous adhesions were present between the intestinal surfaces so that they were matted in nodular masses. In some areas small vesicular nodules were seen over the margins of the neoplasm.

The stomach and intestines, including the rectum, were normal except for the changes just described. The appendix situated retroceally was normal.

The dura was smooth and glistening. No gross changes were found in the brain or its membranes. The brain stem, as well as the cerebellum, was grossly normal. The blood vessels and the nerves at the base of the brain showed no gross changes. There was no neoplasm in the cranial cavity.

*Microscopic Examination.*—Sections from the lesions over the serosal surfaces of the uterus, ovaries, small intestines and mesentery showed essentially similar findings.

There was a more or less thickened layer of loose areolar vascularized connective tissue infiltrated with lymphocytes and plasma cells (fig. 2 *B*). Overlying this were irregular masses and groups of ovoid cells, ranging in type from cuboidal to low columnar, with slightly basophilic cytoplasm and large pale vesicular nuclei. There was a definite tendency toward the formation of small papilliferous projections from the surface growth, also toward the development of an alveolar arrangement, so that one might be impressed with the resemblance to carcinoma (fig. 2 *A* and *C*).

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#### EXPLANATION OF FIGURE 2

*A*, area showing alveolar arrangement of the tumor cells with a supporting framework of connective tissue. From such areas a false diagnosis of adenocarcinoma might be made.  $\times 100$ .

*B*, area from the serosal surface of a loop of the ileum at the margin of the mesenteric attachment. The loose areolar stroma is infiltrated with lymphocytes.  $\times 100$ .

*C*, area showing ovarian cortical stroma with its overlying layer of serosal cells. The transformation from normal to neoplastic cells is seen at the lower center and to the right.  $\times 100$ .

Careful observation, however, revealed the true nature of this tumor. In the larger nodular areas one could differentiate two types of stroma: a denser fibrous type without reticulum and a finer fibrillar form in which a definite reticulum was present (fig. 3). These reticulum fibers surrounded small groups of the tumor cells, acting as a supporting stroma, but did not surround or enter into the individual cells.

In sections in which a continuous surface lining was seen the cells showed rounded-out bulging free surfaces with deep indentations between them, so that the free border acquired a serrate appearance. This appearance is rather characteristic of tumors developing from mesothelium and is strikingly different from the appearance of developing carcinoma. In the sections taken from the growth over the ovary there was a definite transition from normal ovarian serosal cells to neoplastic cells, as demonstrated in figure 2 C.

Where the nodules were larger and more stroma was present, there was a tendency toward infiltration, and more changes were noted in the cell nuclei, more anaplasia was present, and some cells contained two or three nuclei. In the areas where little underlying stroma was present, there was no invasion but more of a tendency to grow away from the surface and to form papillary projections.

An occasional small area showed the cells with a fine fibrillar stroma forming small vesicle-like projections from the serosal surface. These findings were therefore so representative of a growth of the serosal cells that we feel that carcinoma can be excluded.

Mucin stains of these tissues gave negative results. No mucin was present in the peritoneal fluid.

#### COMMENT

Miller and Wynn<sup>3</sup> reported a case in which they noted two types of connective tissue—a well developed fibrous tissue in bands carrying vessels, mostly well formed, and constituting the supporting stroma, and a finer fibrillar type seemingly surrounding the tumor cells, forming a felted network. They suggested that the fibers appeared to be developed from the tumor cells. In their case there was a mucinous ascitic fluid which they stated was secreted by the tumor cells. We are able to confirm the findings of Miller and Wynn in that there are two types of connective tissue present in these tumors. A study of reticulum stains shows one type, minus reticulum, forming the denser and vascular stroma, the other, containing reticulum and forming a network around and between groups of the tumor cells. We cannot confirm their opinion that these fibers arise from and can be followed into the tumor cells.

The denser reticulum-negative stroma makes up the larger part of the nodules present in some areas of the growth.

We are also of the belief that the true tumor cells derived from the peritoneal serosa secrete no mucin. Mucin stains were negative in our case. Miller and Wynn thought that in their case the mucin present in the peritoneal fluid was secreted by the tumor cells. Hamdi and others<sup>4</sup> stated that in their cases ("adeno-coelothelioma") there was no tendency toward generalization and that no metastases were encountered.

3. Miller, R. T., and Wynn, W. H.: *J. Path. & Bact.* **12**:267, 1908.

4. Hamdi, H.; Louthai, M., and Schevket: *Beitr. z. path. Anat. u. z. allg. Path.* **19**:441, 1928.



Fig. 3.—*A*, area from the mesentery stained for reticulum. The reticulum fibers are seen between groups of tumor cells, forming a fine supporting stroma.  $\times 100$ . *B*, higher magnification of a section stained for reticulum, showing the fibers between the groups of tumor cells. It is seen that the individual tumor cells are not surrounded by the reticulum fibers and that these fibers do not extend into the tumor cells.  $\times 400$ .

No metastases were reported by Miller and Wynn. Bender, cited by Miller and Wynn, reported no metastases in his case and assumed that the growth proves fatal before metastases occur. No metastases were present in our case.

#### SUMMARY

A case of mesothelioma (endothelioma) of the peritoneum is reported. Careful search failed to reveal any other tumor in the body. The similarity in structure between this neoplasm and carcinoma may be explained by the ability of the peritoneal cells to assume the role of epithelium and to produce epithelium-like structures. There were two types of connective tissue in the tumor. One was a reticulum-positive supporting structure; the other, reticulum negative, formed the stroma. No mucin was secreted by this tumor.

## PULMONARY INFARCTION AND ATELECTASIS

### Report of a Case Presenting Evidence of a Causal Relationship

BENJAMIN CASTLEMAN, M.D., BOSTON

In a recent paper by Fleischner, Hampton and Castleman<sup>1</sup> it was demonstrated that linear roentgen shadows are produced by platelike foci of atelectasis, healed pulmonary infarcts or interlobar pleuritis. Although mention was made of the fact that both atelectasis and infarction are prone to occur in areas of impaired ventilation and circulation, no definite evidence of any relation between the two was presented. A great deal, however, has been written about the relation of atelectasis to pneumonia, and many investigators, especially Coryllos,<sup>2</sup> have expressed the belief that both lobar and bronchopneumonia develop on preexisting atelectasis. In a paper on the circulation of the lung, Coryllos and Birnbaum<sup>3</sup> showed that the circulation and ventilation of the lung are "parallel functions"; when one is impaired, the other is also decreased. In the case to be presented here there is fairly good morphologic evidence that an infarct may develop in a preexisting atelectatic area.

#### REPORT OF CASE

A 56 year old woman entered the hospital for fecal incontinence and was found to have a tumor of the sigmoid with pelvic extension, diagnosed as carcinoma. A colostomy was performed, but following operation she gradually failed, and on the fourteenth postoperative day she died. A roentgenogram of the chest made on admission showed no evidence of disease. A postmortem tele-roentgenogram (fig. 1 A), taken according to a technic previously described,<sup>4</sup> showed a definite linear horizontal shadow across the lower third of the lower lobe of the right lung. Since this shadow was not present on the preoperative roentgenogram taken nineteen days before, it was interpreted as probably a post-operative platelike atelectasis.<sup>5</sup>

Postmortem examination confirmed the diagnosis of carcinoma of the recto-sigmoid with marked peritoneal extension and also with metastases to the liver. The base of the lower lobe of the right lung was adherent to the dome of the diaphragm by thin fibrous adhesive strands. The left pleural cavity was completely obliterated by old adhesions. No fluid was present on either side. Both lower lobes were slightly congested and subcrepitant. In the upper portions of the upper and lower lobes of the right lung there were characteristic brownish

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From the Department of Pathology and Bacteriology of the Massachusetts General Hospital.

1. Fleischner, F.; Hampton, A. O., and Castleman, B.: *Am. J. Roentgenol.* **46**:610, 1941.

2. Coryllos, P. N., and Birnbaum, G. L.: *Arch. Surg.* **16**:501, 1928.

3. Coryllos, P. N., and Birnbaum, G. L.: *Arch. Surg.* **19**:1346, 1929.

4. Hampton, A. O., and Castleman, B.: *Am. J. Roentgenol.* **43**:305, 1940.

5. Fleischner, F.: *Wien. Arch. f. inn. Med.* **28**:461, 1936.



red, well circumscribed infarcts, each measuring about 2.5 by 3 by 2 cm. Microscopically, these two infarcts showed practically no organization and were almost certainly of very recent origin.

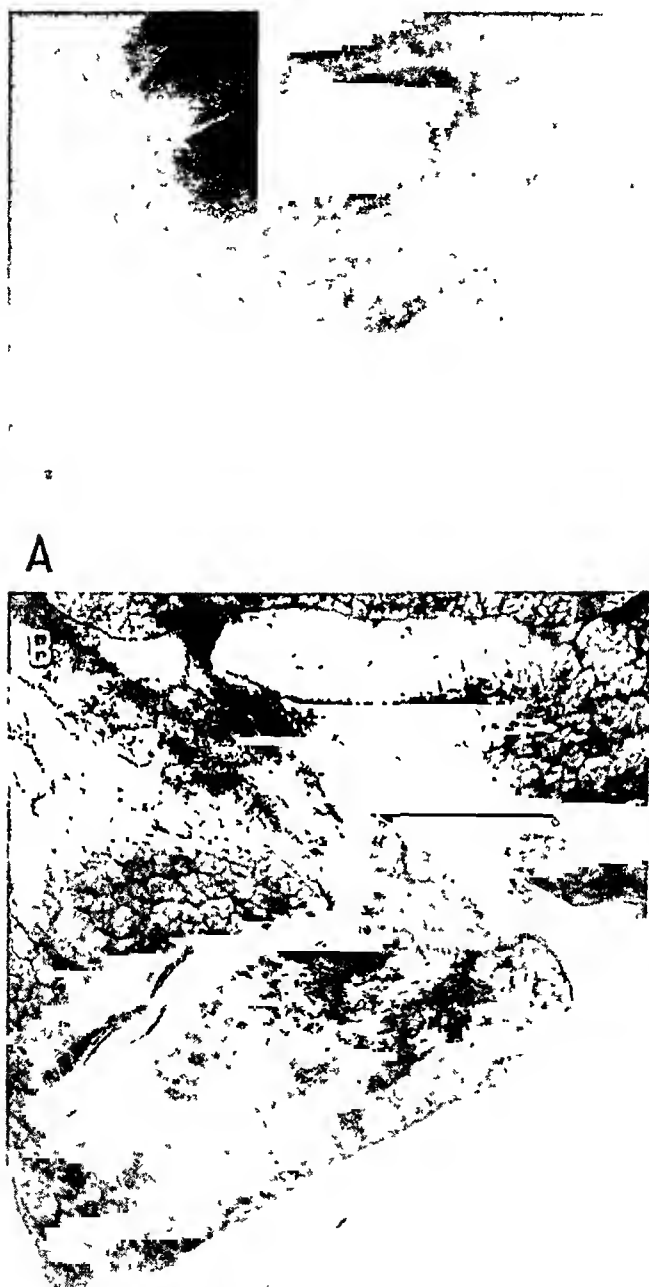


Fig. 1.—*A*, postmortem teleroentgenogram of the right lower lung field showing a homogeneous area of density placed against the lateral pleura. *A* roentgenogram taken nineteen days before did not show this abnormality. *B*, photograph of the lower half of the right lung, showing linear hemorrhagic sulci across the lower and middle lobes.

Across the lower third of the lower lobe of the right lung was a dark red depressed linear area extending from the anterolateral border horizontally across

the whole anterior surface of the lobe to the medial border and continuing along the medial surface in the region of the interlobar septum (fig. 1 *B*). This lesion was 7 cm. long and 0.2 to 0.4 cm. wide. It was deep red and quite firm, and when the lung was injected with 4 per cent solution of formaldehyde through the trachea, the distention of the parenchyma surrounding the linear area exaggerated the depressed cleftlike effect of the lesion. The fact that the latter could not be inflated does not rule out atelectasis, for in most of these cases it is almost impossible to overcome the surface tension of the contiguous alveolar walls. From the gross appearance at autopsy it was felt that the shape and the location of this lesion were characteristic of the platelike focus of atelectasis previously

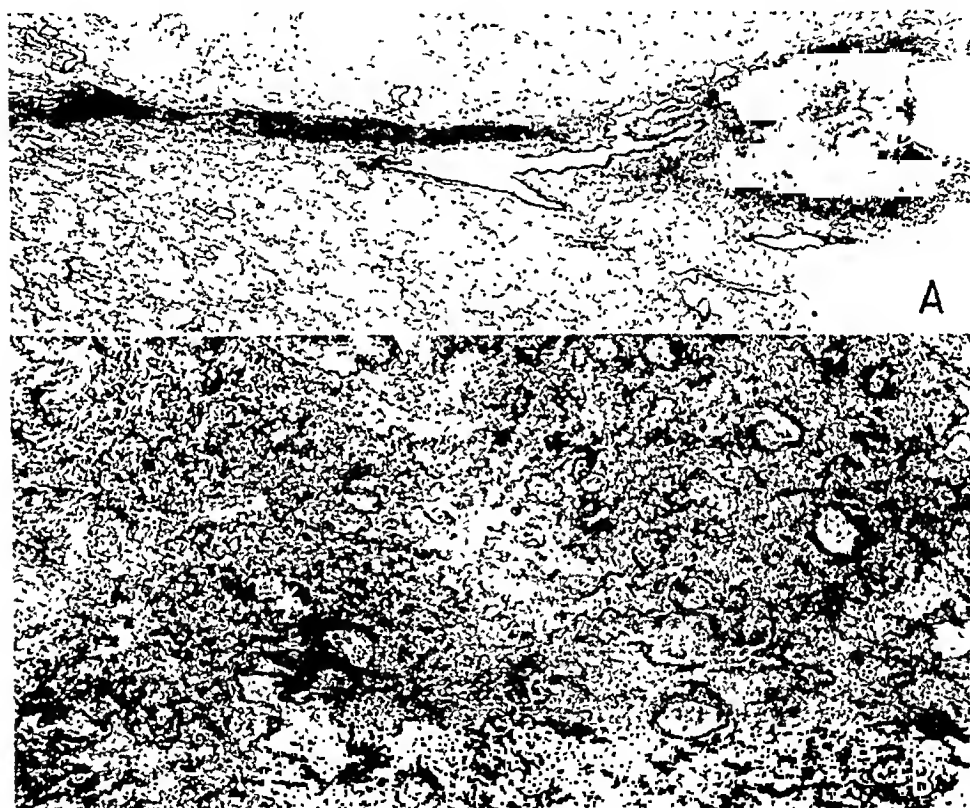


Fig. 2.—*A*, photomicrograph at very low magnification showing the shape of the lesion in the lower lobe (elastic tissue preparation). *B*, photomicrograph at higher magnification showing a recent infarction with destruction of elastic fibers (elastic tissue preparation).

described by Fleischner.<sup>5</sup> The red color and the apparent firmness of the linear lesion, however, were factors against that diagnosis, since atelectasis is usually blue or purple and in the linear form not firm. The other possibility was that it might be a healed infarct, but this could be ruled out by the fact that a healed infarct of this size must have been much larger in its acute stage, and it would have taken at least months to shrink down to its present size.<sup>6</sup> The

6. Castleman, B.: *Arch. Path.* 30:130, 1940.

roentgenogram taken three weeks before death did not show any lesion. When sectioned vertically and perpendicularly to the pleura, the lesion extended into the parenchyma for 3 cm. as a thin rigid band, widest at the pleura and tapering off medially (fig. 2*A*). Microscopic examination of this lesion (fig. 2*B*) showed necrosis, hemorrhagic infiltration, deposition of hemosiderin and shadows of alveolar walls—observations characteristic of recent infarction. The infarction was most severe near the pleura. Definite recent thrombi were present in the pulmonary arteries, both within and just outside the infarct. Slight but definite organization of the peripheral margins of the infarct had already begun, which was evidence that the lesion was probably between one and two weeks old. The surrounding alveoli were partially atelectatic and in places filled with fluid.

On the anteromedial surface of the middle lobe of the right lung, extending obliquely downward and somewhat medially, was another very similar linear band, about 5 cm. long and 0.4 cm. wide, which was histologically the same as the one just described.

The important and somewhat intriguing feature of this case is to explain the genesis of the horizontal bandlike shapes of the recent infarcts in the lower and middle lobes of the right lung. In a discussion on the shape of pulmonary infarcts Hampton and Castleman<sup>4</sup> stated, "The shape of the infarct is dependent entirely upon the shape of the part of the lung it involves." The shape of the lesion in question is definitely that of platelike atelectasis, since there is no other recent process (it must be recent since a preoperative roentgenogram did not show it) that could involve a whole lobe over such a narrow area. The shape, the distribution and the size of the lesion are that of atelectasis, but the microscopic picture is that of infarction. Could the infarct have developed in a preexisting platelike focus of atelectasis? The presence of atelectasis around the lesion might be conceived as being part of the original atelectatic focus that had not become infarcted, but this evidence is not too valid since it might well have resulted from the pressure of the infarct on the adjacent alveoli, such as that seen around any area of consolidation.

The presence of two typical infarcts in this same lung in addition to the lesions in question is evidence that emboli were entering this lung, and any focus of impaired ventilation and circulation would most certainly be vulnerable to infarction if an embolus happened to reach that area. Whether the atelectatic focus was in any way a "drawing card" for the embolus is questionable, although Nissen<sup>7</sup> showed experimentally that emboli introduced into a peripheral vein will go regularly and without exception to that lung which has previously been collapsed. These experiments in rabbits were concerned with collapse of a whole lung, and it is doubtful whether a relatively small atelectatic focus could have a similar effect.

It has been fairly well established that bland pulmonary infarction does not occur in a normal lung, the usual prerequisite being pulmonary congestion. Although this case illustrates that infarction may develop in a preexisting focus of atelectasis—a condition in which both ventilation and circulation are impaired—it is not suggested that atelectasis

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7. Nissen, R.: *Arch. f. klin. Chir.* **167**:567, 1931.

plays a role in the development of the usual infarct. Infarcts have often been found in atelectatic lobes, but a definite causal relation, although intimated, has never been proved. The unusual shape of the infarcts in the case presented affords more evidence of this relationship.

#### SUMMARY

A case is presented in which two linear recent pulmonary infarcts were observed at autopsy. This unusual shape for a recent infarct is given as evidence that the lesion developed on a preexisting platelike focus of atelectasis. The question of atelectasis as a predisposing factor for embolism and infarction is discussed.

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# General Reviews

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## EFFECTS OF RADIATION ON NORMAL TISSUES

SHIELDS WARREN, M.D.

BOSTON

*(Concluded from Page 139)*

### X. EFFECTS ON ORGANS OF SPECIAL SENSE

#### THE EYE

The natural apprehension lest the eye should be injured when radiant energy was directed to neighboring structures led to early consideration of its sensitivity. When roentgen rays and radium were first utilized, there were even claims that the blind could see by means of them. Animal experimentation has been of necessity the chief source of information as to the effects on the normal eye. There is little evidence dealing with the relation of the dose to the reaction of the human eye.

The severe destructive action of roentgen rays on the structures of the anterior segment of the eye, namely the conjunctiva, the cornea and the iris, in long exposures was described by Chalupecky in 1897. Although there is little difference in the sensitivity of these three structures, they react in this order. Conjunctivitis and keratitis are useful indications of the strength of the radiant energy, especially in the early reports, in which the factors of radiation are often not stated. The reaction is elicited by somewhat smaller doses than are required to produce dermatitis, and the latent period is generally shorter. This sensitivity of the outer parts of the eye is greatest in newborn animals and lessens with maturity. Conjunctivitis has been described after slight irradiation of the eye of a kitten (Belley), while in rabbits 2 to 4 weeks old conjunctivitis developed only after exposure to moderate to heavy radiation, depending on whether it was not or was filtered (Frogé). In the experiments of Birch-Hirschfeld (1904 a) the latent period seemed to depend more on the dose than on the age of the animal. The most marked pathologic changes were seen in the adult rabbit which had received 20 Holz-knecht units in thirty minutes and was killed thirty days later. After a latent period of sixteen days, the following gross changes gradually appeared: mucopurulent conjunctivitis, interstitial

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From the laboratories of pathology of the Harvard Cancer Commission, New England Deaconess Hospital and Pondville State Hospital for Cancer, and the department of pathology of Harvard Medical School.

keratitis, contraction of the pupil, and bleaching and hyperemia of the iris. The cell changes in the conjunctiva were marked in degree, especially in the region of the conjunctival fold, and irregular in distribution. There were desquamation of epithelium and different stages of degeneration with attempts at regeneration. The nuclei stained poorly; some of them were long and shaped like a biscuit or a dumbbell; atypical mitotic figures were present. Leukocytic infiltration appeared most marked around the hyperemic vessels in the region of the limbus, and the vascular endothelium was swollen. In the anterior layer of the cornea there was quite sharply outlined necrosis. The epithelium was in part desquamated, and in some places there was only a single layer of cells with elongate nuclei. At the periphery of the cornea, the epithelial cells were greatly swollen, their nuclei were irregular in shape and sometimes duplicated, and mitotic figures were more numerous. There was some general edema, as well as leukocytic infiltration. The reaction might last several months. One rabbit on whose eyelid an ebonite tube containing 20 mg. of radium-bromide was allowed to remain for five hours showed, after a latent period of sixteen days, conjunctivitis and clouding of the cornea, which had not completely subsided eighty-nine days later. At this time the microscopic section showed moderate leukocytic infiltration of the conjunctiva and in the cornea moderate dilatation of interfibrillar spaces, irregularity of corneal corpuscles and questionable infiltration by leukocytes. The anterior chamber was filled with fibrinous exudate. Regeneration appeared first in the cornea of the rabbit, somewhat earlier than in the conjunctiva after 2 erythema doses, and might be seen for a long time afterward (Rohr-schneider)—in 1 rabbit, two hundred and fifty-eight days after 1.5 erythema doses.

Jacoby irradiated adult rabbits with doses expressed as erythema doses for man. In one eye given 2.5 erythema doses in one hundred and fifteen minutes soon hyperemia of the conjunctiva developed, and later on abundant mucopurulent conjunctivitis. Epilation occurred in ten days and extensive radiodermatitis in three weeks. Twenty days after exposure the cornea showed flecks that finally merged to cover it completely. Dendritic erosions formed which subsided entirely after nine months. Another eye receiving 3 erythema doses showed epilation after two weeks and keratitis after three and a half weeks, subsiding in three and a half months. Five erythema doses given in three doses over a period of eleven weeks produced marked swelling of the lids and conjunctivas, abundant secretion and severe keratitis with ulceration, which continued undiminished five and a half months. At the end of this time microscopic examination revealed degenerative changes in the epithelium of the conjunctiva and the cornea, leukocytic infiltration,

especially around the vessels in the vicinity of the limbus, and swollen, vacuolated endothelium. Jacoby concluded that the epilation dose for the rabbit is approximately 2 erythema doses for man, and the injurious dose for the cornea is approximately 2.5 doses. The latent period for the reaction of the tunics was from two to five weeks.

The results of most investigators are in conflict with those of Rados and Schinz, who claimed 19 human erythema doses had no effect on rabbits' eyes, while 17 doses caused loss of hair from the eyelids after a latent period of three weeks and slight conjunctivitis and keratitis after four weeks. Twenty-one and 26 erythema doses produced total loss of skin from both eyelids and marked conjunctivitis and keratitis.

Cataract is the most important injury resulting from irradiation of the young or partly developed eye. It is produced by exposure to radiation (moderate or heavy, depending on the factors of time, filtration, and so on). The striking contrast in sensitivity between the lens of the young animal and that of the old one is explained by the fact that there is still active cellular proliferation in the young lens (Tribondeau and Belley). The equatorial and posterior cortical regions which are the most rapidly proliferating are said to be most affected by radiation (Bossuet). Lenticular opacity was found in a little over half of the fetuses whose mothers had been irradiated, even though in some instances the abdomen was shielded (von Hippel, 1907). Milroy observed the opposite in the chick embryo: a marked sensitivity of the retina as compared with the lens. Among the earliest reports are those of experiments on newborn kittens (Belley; Tribondeau and Belley; Tribondeau and Récamier). The superficial parts of the eye were found to be damaged by soft roentgen rays, while the lens was most affected by medium and hard rays. It was claimed that as little as five minutes' exposure to roentgen rays would cause a certain degree of opacity of the lens of very young kittens (Tribondeau) and almost complete vascular and granular degeneration of the lens of a 3 day old kitten resulted from the application of moderate unfiltered soft radiation (Tribondeau and Récamier<sup>1</sup>). Gross changes in the lens were noticed thirty-three days (Tribondeau and Belley) after treatment with soft and medium rays (Tribondeau and Récamier), moderate radiation (Belley; Tribondeau and Belley) giving microscopic evidence of injury fifteen to twenty-three days after exposure (Belley). The earliest abnormalities have been described as flattening and degeneration or complete disappearance of the lenticular epithelium on the anterior surface. Some hypoplastic thickening of the epithelium may occur. The lenticular fibers become enlarged and tortuous, owing to vacuolar and granular

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1. The radiation treatment consisted of six exposures of ten minutes each at 10 cm. distance given at the rate of three a week.

change. The greatest effect was seen in the equatorial zone. Frogé produced cataracts in young rabbits with moderate to heavy radiation, filtered as well as unfiltered. The lenses became disproportionately small as the animals grew. Peter described cataracts in rabbits 5 to 10 months old, developing between forty-five and three hundred and twenty-five days after treatment, depending on the strength of the dose. The doses were 1 to 10 erythema doses (150-180 kilovolts; 1 erythema dose equals 600 r). Clouding of the lens occurred at ninety to two hundred and sixty days after exposure to from 160 to 640 milligram hours of radium. Microscopic examination showed vacuoles chiefly in the posterior part of the lens.

The lens of the adult rabbit is very resistant. No effect was observed up to six weeks after thirty to sixty minute exposures (Tribondeau and Lafargue). A similar resistance was observed by Jacoby after exposure of adult rabbits' eyes to 3 human erythema doses for sixteen minutes or 2.5 doses for one hundred and fifteen minutes, or 5 doses in three sessions over a period of eleven weeks. In the last instance the normal gross appearance was confirmed by microscopic examination five and a half months after the final treatment. Nor was the lens affected by placing 20 mg. of radium bromide on the eyelid for periods of two to six hours (Birch-Hirschfeld, 1904 b). Rados and Schinz described normal lenses in rabbits after 26 human erythema doses.

Bossuet made careful studies of the lenses of fetal, young and adult animals, using dogs, rabbits and guinea pigs, and concluded that a lens of any age may be injured by roentgen rays but that the effect is much greater in the immature lens. For his experiments on adult animals he used 2 rabbits and 2 guinea pigs. The rabbits were given forty-five minute exposures at 5 cm. distance, and the guinea pigs were given single exposures of forty-five and ninety minutes at 5 cm. The rabbits were killed two and thirty-one days afterward and the guinea pigs at ten and eleven day intervals. The rabbit killed after two days showed no change, but the other 3 animals had a similar reaction of the lens, consisting of degeneration of the capsular epithelium as well as cellular proliferation.

Meisner produced cataract in an adult rabbit by introducing 0.68 millicurie of radium emanation into the vitreous. Eight days later there was clouding of the vitreous, and twelve days later, slight blurring of the aqueous humor and fine precipitation on the anterior capsule of the lens. After sixteen days there was husklike opacity of the posterior capsule. The lens was totally opaque at the end of one month.

Thorium-x injected into the eyes of adult rabbits has produced suggestive but inconclusive results. Abelsdorff injected thorium-x into the aqueous of the eyes of adult rabbits in amounts equivalent to



Radium placed on the eye or within the eye will injure the retina and the nerve. Birch-Hirschfeld (1904 b) described rather vague degenerative changes in the retina and the ganglion cells of an adult rabbit, together with myelin degeneration of some of the fibers of the medullary rays (demonstrated by Marchi preparations). Twenty milligrams of radium bromide in an ebonite tube had been placed on the eyelid for two hours, and the microscopic examination showed a normal retina at the end of one hundred and twenty days. Eighty-nine days after a five hour exposure to the radium tube, the changes, though early, were slightly more definite. Some of the ganglion cells of the retina were vacuolated and the nuclei shriveled. A rather marked degeneration of myelin was seen in the optic nerve and the medullary rays. The same kind of change in the optic nerve, although possibly greater in degree, was found ninety-seven days after a six hour exposure to 20 mg. of radium bromide. The isolated retina of the adult rabbit first shows indications of injury from twenty to forty-five minutes after exposure (Birch-Hirschfeld, 1904 b). Tribondeau and Lafargue (1908) took issue with the findings of Birch-Hirschfeld (1904 b) and claimed that the changes which he described were within normal limits. Furthermore, two months after even larger doses they were unable to observe changes in the retina. Complete degeneration of the retina, degeneration of the medullary sheaths of the optic nerve, as well as opacity of the lens of an adult rabbit's eye, followed introduction into the vitreous of tubes containing 0.68 millicurie of radium emanation (Meisner).

Eichbaum injected a solution of radium chloride into the eye of a rabbit. One-half millicurie produced no changes. Four weeks after 1 millicurie had been given there were fibrinous and fibrous hyalitis, chorioretinitis and neuritis. One and five-tenths millicuries caused inflammation of the whole bulb, especially of the ciliary body, for one to four months after its administration.

Abelsdorff described disappearance of the layer of rods and cones, edema and loosening of the swollen layer of nerve fibers, as well as degeneration in the anterior portion of the optic nerve, after he had injected into the eye an amount of thorium-x equivalent to 0.1 mg. or more of radium bromide.

The pigment of the iris of the young animal is affected by moderate to heavy radiation and that of the adult by heavy radiation (Stargardt). The pigment granules become clumped in the chromatophores, and these disintegrate and finally disappear. The same type of change

appears in the chromatophores of the choroid after the latter has been heavily irradiated. Radiation sufficient to produce changes in the chromatophores will have produced more or less severe iritis with congestion, edema, petechial hemorrhages, posterior synechiae and later atrophy of the iris, including the ciliary muscle.

The most obvious expression of the inhibitory influence of radiation on the growing eye is the narrowing of the palpebral fissure. It is not entirely clear from the early reports how much radiation is necessary to retard growth (Belley). After exposure to moderate radiation, development does not stop altogether but continues at an apparently even, though much slower, rate.

Congenital abnormalities of the eye have been described by various authors. Three or more weeks after treatment of the pregnant mother, the animals are usually premature or at least stillborn and cadaverous. The radiation producing these effects has been moderate or heavy. Defects of the lids are a quite constant finding. Microphthalmos, coloboma (von Hippel, 1906) and hemorrhages into both chambers, as well as opacities of the lens, have been described (Bossuet; von Hippel, 1906, 1907). In most instances it is not possible to be sure which defects result primarily from the radiation directed to the structures of the eye and which are secondary effects.

Some of the discrepancies and negative findings (Roselli) reported may be laid to the making of observations too soon after the completion of radiation treatment.

#### THE EAR

The effects of radiation on the ear are at present but ill understood. This is natural since most of the diseases of the auditory apparatus itself are not susceptible to treatment by radiation and there are few lesions in the vicinity of the ear the treatment of which by radiation would lead to injury of the organ. Practically all of the early experiments are largely vitiated by the fact that extensive trauma was induced by the method of applying the radium or the radioactive substances; much of the reaction encountered was due probably to the implantation rather than to the effect of the radiation. Such are the early experiments of Ewald and Marx. These experiments present much detailed description of the behavior of the few animals and birds treated and of the lesions found. Chilow placed radon in glass in a tympanic cavity in each of 3 cats. There was marked hyperemia of the region; the cells of the organ of Corti were represented by a shapeless mass; the scalae were infiltrated by leukocytes, and there was an inflammatory picture through

most of the region. As Desjardins pointed out, these experiments were ill controlled.

Marked hyperemia of the middle and inner ear has been produced by roentgen radiation of unspecified amount (Thielemann). This hyperemia was so marked as to produce perilymphatic hemorrhage in the inner ear. Serous or seropurulent exudation developed in the middle ear.

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## XI. EFFECTS ON ENDOCRINE GLANDS

The sketchy histologic data bearing on the sensitivity of the pituitary gland to radiation are in marked contrast to the great mass of relative clinical reports. Animal experimentation has given practically all the information at hand, and this is inadequate, especially because of the one-sided interest of most workers: Seldom have the factors of radiation and the physiologic and histologic observations been presented with equal clearness. In general the changes described are of a minor nature. Several studies on animals have demonstrated congestion, edema and "degeneration" of the anterior lobe after moderate and heavy irradiation with roentgen rays or radium. There is a general impression that the eosinophils are principally affected. They are often said to be decreased in number, although the cells are not usually counted. Degenerative changes in the pars nervosa and the pars intermedia have been described only after heavy irradiation.

Suppression of growth and sex function and slight histologic changes in the pituitary gland have been reported by several writers. Lawrence, Nelson and Wilson treated albino female rats 30 to 40 days old with roentgen rays (180 kilovolts) directed to the pituitary gland through a hole 1 cm. in diameter in a lead shield. It was found that 2,080 r in two doses a few days apart was the smallest amount of radiation which would affect, with any degree of certainty, either the general physical condition of the animal or the histologic structure of the pituitary gland. The changes in this gland were most consistent eighteen to twenty-two days after treatment. At later periods the gland appeared histologically normal. The functional effects closely resembled those following subtotal hypophysectomy: loss of body weight, hypoplasia of the pituitary gland, ovaries, thyroid gland and adrenal glands and loss of ovarian activity. In three reports changes in the pituitary gland were discussed which were so slight as to be questionable: Epifanio and Cola thought the growth of rabbits (both young and adult animals were used) was accelerated with 25 per cent of an erythema dose and inhibited with larger doses. Obvious suppression of growth and development was observed by Brunner in a kitten after 4 erythema doses given in one treatment and in dogs after 2.5 erythema doses given in four treatments three to four days apart, and by Fraenkel and Geller in bunnies after 75 to 50 x units. Rather more definite histologic changes were described by Podljaschuk (1927); degeneration and atrophy were present in the

anterior lobes of dogs fifty-eight days after a 130 per cent erythema dose and glial proliferation in the posterior lobes seventy-two days after this treatment.

Other workers using comparable doses found no change in the pituitary gland to correspond with the physiologic changes.

Fehr gave sexually mature rabbits massive doses—3,300 r to 10,160 r (180 kilovolts). The dose reaching the pituitary gland was estimated as 80 per cent of the dose reaching the skin. Massive doses produced loss of body weight and atrophy of the genitalia, whereas, if the doses were fractionated, the animals continued to gain in weight. The pituitary glands were examined four to six weeks after irradiation. Martinalli reported loss of body weight and pathologic changes in the ovaries and uteri of mature rabbits resulting from exposure to radiation, although the pituitary glands of these animals remained normal.

Contradictions found from one report to another result partly from difference in the interpretation of observations. The findings of Ghilarducci are especially hard to reconcile with other reports. Irradiation of young rabbits produced death, with complete destruction of the anterior lobe of the pituitary gland and skeletal deformity, but the ovaries and other endocrine organs were normal.

In some reports the pertinent data are chiefly histologic. Thus Cannavo and Beninato described "marked change" in the pituitary glands of rabbits dead of cachexia fifteen to twenty days after the administration of 700 to 1,000 r in three doses. This "change" consisted of swelling of eosinophils, without reduction in their number, and reduction of chromophobes. The pituitary glands of adult dogs showed varying degrees of atrophy and degeneration of the anterior lobe after 85 to 90 erythema doses (Podljaschuk, 1928). A reduction in the number of eosinophils in the pituitary glands of guinea pigs was described by Franck thirty-eight to seventy-seven days after large doses of roentgen rays. The metabolism of these animals was studied by Okkels and Krogh in connection with changes in other endocrine glands. The results were not outstanding.

In some instances, irradiation of the pituitary region has been without effect. Del Buono, using adult dogs, found no alterations in the pituitary gland or other organs six months to a year after nearly an erythema dose had been directed to the pituitary region. Kotz, Elward and Parker treated rabbits with a single dose of radiation (200 kilovolts) in amounts which were tremendous as compared with therapeutic doses. There was no demonstrable change in the weight of any animal or in the size or

structure of the gland three to seven weeks after treatment. The normal ratio of the eosinophils, basophils and chromophobes of the anterior lobe was maintained and there was no degeneration.

Selle, Westra and Johnson (1935, 1938) studied the physiologic effects of irradiation of the pituitary gland on 7 depancreatized dogs. The treatments were given several weeks after the dogs had been operated on; 2 dogs received a single treatment, and 4 dogs had two treatments three to four weeks apart. The estimated doses reaching the pituitary gland at 140 kilovolts were 1,500 r and 2,025 r for a single dose and 2,360, 2,045, 2,165 and 3,170 r for a total of two doses. The two treatments were given about three weeks apart, and the animals were killed three to four weeks after the single treatment or after the last treatment. Five animals (1 dog was not examined) showed some sort of mild change in the pars tuberalis such as numerous eosinophils after 2,045 r, edema after 2,165 r or "slight degeneration" after 2,025 r and 1,500 r, and "marked degeneration" after 3,170 r. Degeneration in the pars nervosa and the pars intermedia was slight after 2,165 r and marked after 3,170 r. Apparently there was no actual necrosis.

The only reference to the histologic effect of radiation on the human pituitary gland is a remark by Friedman that he had seen a normal pituitary gland after a total depth dose of 20,000 r given over a period of several years.

The clinical effects which have been described after roentgen treatment of the diseased pituitary gland as in the case reported by Cushing, are not elucidated by the studies on the normal animal's pituitary gland.

After due allowance has been made for the rough methods of estimating injury of cells, the difference in size of laboratory animals, methods of treatment, and so on, it seems that only heavy radiation has a very disturbing effect on the normal pituitary gland and there is little chance that therapeutic radiation affects it at all. It is possible that roentgen rays may have an entirely different effect on the diseased gland.

#### THE PINEAL GLAND

My associates and I have found no data bearing on the effects of radiation when this is directed at the pineal gland.

#### THYROID AND PARATHYROID GLANDS

A large amount of data has been made available as to the effects of radiation on tumors of the thyroid gland and on toxic goiter. This review, however, is restricted to the effect of radiation on normal tissues, and the data in this connection are relatively few. The thyroid gland may be considered fairly resistant (Walters, Anson and Ivy, 1932).

The earliest observation of an irradiated thyroid gland is that of Murray, who noted diffuse growth of the interalveolar connective tissue, possibly as a result of the parenchymal atrophy following irradiation.

Many of the early experiments were inconclusive but tended to emphasize the resistance of the thyroid gland (Krause and Ziegler). However, in many animals the skin over the thyroid gland showed no change; so the irradiation of the gland must have been slight.

By direct implantation of radium, Bower and Clark, using 12.5 milligram radium needles, produced necrotic lesions in dogs after doses varying from 25 to 169 milligram hours. Up to the third week a yellowish zone of necrosis bordered by a narrow reddish hemorrhagic band surrounded the site of the needle. Microscopically, the picture was one of necrosis and hemorrhage; the central material was completely amorphous but merged into a peripheral zone of necrosis resembling that seen in an early infarct with shadowy outlines of preexisting structures. In the outer border of this zone were remnants of fragmented nuclei, lymphocytes, a few plasma cells and occasional polymorphonuclear leukocytes. Surrounding this was a zone of hemorrhage showing only slight degenerative histologic changes, such as swollen cuboidal epithelium with deeply stained nuclei and scanty colloid. Beyond this, the thyroid tissue was essentially normal. After about three weeks, organization and healing began as a result of peripheral fibroblastic proliferation. There was some phagocytosis of the necrotic material, and occasionally calcification occurred.

The thyroid gland of the rabbit, although physiologically labile, requires very heavy radiation to affect it (Coulaud). Heavy roentgen radiation produced slight alteration in follicular size and some nuclear degeneration but no fibrosis. Guinea pigs were exposed to 50 to 400 milligram hours of radium radiation at a distance of 1 cm., and their thyroid glands were removed from one to fifty-eight days later (Eckert, Probstin and Galinson). The glands of the treated animals could not be told from those of the control group. After three fourths of their thyroid tissue had been removed, the guinea pigs were exposed to 100 to 150 milligram hours of radium and killed from one to fifty-eight days later. These glands showed some depletion of colloid and some lymphocytic infiltration.

The claim has been made (Florentin and Watrin) that roentgen treatment can produce in guinea pigs a hyperplasia similar to that seen in pregnancy, with increase in size of some of the cells and some mitotic activity. The nuclei described were not unlike those sometimes seen in slight spontaneous hyperplasia.

Pfeiffer failed to find changes in the thyroid glands of normal dogs after these had been given roentgen radiation but did obtain shrinkage of the thyroid glands in over half the patients with goiter who had been treated with doses large enough to redden or blister the overlying skin. Similarly, Rave observed no histologic changes in the thyroid glands of rabbits with doses of from 12 to 36 Kienböck units (about 1 to 3 erythema doses); in patients with goiter he found diminution of lymphoid tissue but no change in epithelium or colloid.

Young rats are more susceptible than adult rats, 1,000 r shifting the epithelium from cuboidal to flat and increasing the colloid storage (Viethen).

The claim is made by Takayama that he produced changes in the Golgi apparatus of the thyroid parenchymal cell with 35 r. This I cannot accept.

Heavy roentgen radiation produced hyperemia of the thyroid gland, followed by cellular degeneration and finally necrosis (Redaelli).

Slight hyperemia progressing to slight nuclear degeneration as the dose was increased to 1 erythema dose was observed by Tsuzuki.

Walters, Anson and Ivy (1931) irradiated dogs at 170 kilovolts, giving four doses of 380 r each from seven to fourteen days apart. In man three such doses on successive days resulted in a second or third degree burn. The dogs received from one to three series delivered to the region of the thyroid gland at varying intervals of time. Between some series, portions of the thyroid gland were removed. In some of the more heavily irradiated animals, thickening of the capsule had occurred. The irradiated animals showed a slightly greater tendency toward hyperplasia than normal dogs. The only change noted histologically was the capsular thickening, which developed only in those animals with burns and edema of the skin. This offers marked proof of the resistance of the gland.

Zimnitsky, Baskina and Devirz irradiated 15 male rabbits at 140 kilovolts giving 550 r daily in from three to ten daily doses. The animals were killed from one day to forty days after treatment. The thyroid glands of some of them showed slight hyperemia; those of others, slight vacuolation of the cytoplasm; with larger doses there was some coarsening of the mitochondria. Up to a total of 10 erythema doses the structure of the thyroid gland was practically normal. Possibly, there was a slightly higher epithelium lining the follicles than in the normal gland. Isolated cells or cell groups were sometimes affected without change in the parenchyma as a whole. The roentgen rays may stop for a time the production of colloid, so that it may



largely disappear. There were some vascular and connective tissue changes, with a slight increase in the stroma after some time had passed.

Almost no information is available as to radiation effects on the parathyroid glands. Bower and Clark reported no changes in their dogs, but probably the radium needles in the thyroid gland were not close enough to have an effect. According to Zimmern and Battez, sufficiently heavy radiation to produce atrophy of the thyroid gland caused atrophy of the parathyroid glands as well. In the experiments of Walters, Anson and Ivy, after heavy irradiation 1 dog showed hyperplasia of the parathyroid glands, with some fibrosis and capsular thickening. In 2 other dogs the radiation prevented compensatory hyperplasia following removal of two glands.

#### THE THYMUS GLAND

For the effects of radiation on the thymus gland, see chapter III.

#### THE ISLANDS OF LANGERHANS

For the effects of radiation on the islands of Langerhans, see chapter IV.

#### THE ADRENAL GLANDS

Interest in radiation effects on the adrenal glands has come about from two sources: first, the desire to influence various types of pathologic change associated with hyperfunction or hypofunction of these glands, and, second, concern as to the changes that might be induced in an adrenal gland as a result of its exposure to incidental radiation in the course of the treatment of lesions in adjacent organs. Thus a number of the earlier reports, practically all of which are unsatisfactory, deal with the roentgen treatment of Addison's disease. An example of the inadequacy of much of this early accumulation of data is Wiesner's case of Addison's disease, in which improvement followed roentgen treatment although later the patient failed and died. Very similar is the case reported by Golubinin in which a 27 year old man with Addison's disease was markedly improved after fifty small exposures to roentgen rays in seventy days. No follow-up of this case is given.

Some of the early experiments relating to functional aspects seem rather fanciful, as that of David and Hirsch, who claimed that irradiation of isolated adrenal glands with 1 erythema dose resulted in a decrease of the content of epinephrine and that irradiation with 25 per cent of an erythema dose increased the production of epinephrine. The data as presented do not inspire confidence in the results.

An early attempt at hormonal assay of irradiated adrenal gland tissue was carried out by Eisler and Hirsch, who irradiated the whole bodies of rats with 150 to 200 Kienböck units in eight to ten days. An extract of the irradiated adrenal glands was injected into the blood stream of 9 rabbits, and in 4 there was a transient elevation of the blood pressure greater than that produced by injection of an extract from nonirradiated adrenal gland.

It is unusual to find first rate evidence of injury of the adrenal glands as a result of therapy. But that in the case of Smithies is probably good. A healthy 58 year old man received extremely heavy radiation for a supposed tumor of the dorsolumbar part of the spinal column. A month later symptoms of Addison's disease developed, and the patient died soon afterward. There was no microscopic study. On the other hand, it is hard to believe that 30 per cent of an erythema dose as measured to the adrenal glands of a 58 year old woman could have resulted in almost complete fibrosis of the cortex six weeks later, as described by Stephan.

An adequate critical review of the clinical and physiologic aspects of irradiation of the adrenal glands has been made by Desjardins. The little concrete information which one finds is based on histologic observations in animal experiments. Early investigators (Harvey; Strauss; von Decastello) found congestion or hemorrhage or both in mice, guinea pigs and rabbits, which were usually subjected to fairly heavy doses of soft roentgen rays. Atrophy of the adrenal glands in mice as a result of exposure to radiation is an unusual finding which has not been confirmed (von Decastello). Destruction of adrenal tissue has been produced by direct implantation of radium (Wislocki and Crowe). Lacassagne and Samssonow embedded unfiltered tubes containing 4.5 to 10 millicuries in the adrenal glands of rabbits. At death, from five to thirty-one days afterward, there was complete necrosis of these glands except in 1 instance in which a small amount of the glomerular layer persisted. Smaller doses of platinum-filtered emanation destroyed the medulla, leaving some of the cortex intact.

More detailed experiments on the effect of roentgen rays have brought out three points of interest: (1) a certain degree of sensitivity of the cortex; (2) a high degree of resistance of the medulla; (3) the difficulty of distinguishing minimal changes, especially in the cortex.

The great variability in the appearance of the normal cortical cells, chiefly with regard to the lipid content of the cytoplasm, adds an element of uncertainty regarding the minor changes seen in the irradiated gland. The descriptive term "degeneration" usually applies to the amount of cytoplasmic fat and the degree of pyknosis. Alterations of lipid content, usually a decrease, have been described frequently.

However, after well controlled studies, Engelstad and Torgersen were unable to form any definite opinion on this score. Tsuzuki regarded reduction of lipid as the earliest change. This appeared immediately after 32 per cent of an erythema dose<sup>1</sup> had been given and lasted for at least ninety-six hours. Twenty-four hours later there was degeneration of the medullary cords. Similar reduction of fat was noted after the administration of 48 per cent and 64 per cent of an erythema dose. Animals exposed to radiation continuously until death showed reduction of fat after sixty minutes (32 per cent of an erythema dose) and hyperemia and "degeneration" of medullary cords after ninety minutes (48 per cent of an erythema dose). In general at death the irradiated organ was slightly enlarged and often hyperemic. Holfelder and Peiper noticed diminution of lipid in the adrenal glands of guinea pigs after small doses around 80 per cent of an erythema dose. There was slight vacuolation of the cells, as well as irregularity in the size and the staining properties of the nuclei. Some irregularity in the distribution of reticular pigment was seen. All of the changes were present in reticular and fascicular zones; the glomerular zone remained normal. One animal that received 160 per cent of an erythema dose on each of two fields died in four days without macroscopic or microscopic changes in the irradiated gland.

More severe changes follow exposure to heavy radiation but bring out more clearly the absence of any close correspondence between cytologic change and dosage. These changes reflect the difference in sensitivity of animals of the same species and age (Holfelder and Peiper) and a lack of uniformity of reaction from cell to cell of a given organ (Engelstad; Engelstad and Torgersen). The variability of changes due to radiation cannot be reiterated too often. Engelstad was unable to demonstrate any appreciable changes in the adrenal glands of rabbits with doses under 1,500 r, a dose which approaches the skin's tolerance, although there was slight hyperemia of both cortex and medulla twenty-eight days after 1,000 r had been given. The first week after the application of 1,500 r, the adrenal glands appeared normal, but later mild degenerative changes appeared. Larger doses (3,000 r) caused pronounced degeneration of cortical cells initiated fifteen days later and reaching a maximum in the second month. Hyperemia and lymphocytic infiltration of varying intensity were associated with the degeneration. In 1 instance small abscesses formed. The injury seemed to be greatest in the zona fasciculata and the zona reticularis and no definite changes were found in the medulla. Slight fibrosis was present forty-six days after the administration of 3,000 r in a single dose.

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1. All the doses given are those calculated for the adrenal glands.

In supplementary experiments on a large number of rabbits, the most constant changes were found two to six months after irradiation of the organs. Treatment consisted in the administration of 2,200 r or 2,500 r in one sitting (4 by 6 cm. field, with some animals being irradiated by anterior and some by posterior routes; 175 kilovolts; 20 cm. distance). The animals were killed mechanically from four to six months later, with the exception of 6 that died fourteen days to six months after treatment. Four degrees of change were described, not all of which are entirely clearcut to the reader. The earliest reaction consisted of hyperemia. The mildest cell change admittedly could not always be distinguished from variations of the normal gland. Clearcut degeneration accompanied by more or less pronounced hyperemia was first seen six days after irradiation with 2,200 to 2,500 r, evidenced by more abundant cytoplasm and poorly stained nuclei with a dark perinuclear zone. The greatest and most constant injury, consisting of marked dissolution of cells and loss of structure, occurred at the end of three months. In some instances it was seen as late as six months. The glomerular zone was less affected than the others, and there was no bona fide change in the medulla.

Cortical necrosis of rabbits' adrenal glands, examined within forty-six days after the last treatment, followed saturation of the organs at various doses for a week or a week and a half by the method of Kingery. The irradiation of the animals was sufficiently severe to cause death of some from intestinal disturbances (Grabfield and Squier).

Rogers and Martin exposed adrenal glands of 16 dogs directly to unfiltered roentgen rays. Graded doses of from 3.5 to 32 erythema doses were given. The capsule was described as thickened in each instance. Ten erythema doses produced some extension of fibrous tissue into the cortex. This was marked in the cortex after 14 to 19 erythema doses, but only slight in the medulla. One dog died twenty-four days after receiving 32 erythema doses and showed gross edema, complete disorganization of the gland and thrombosis of the lumbo-adrenal vein.

While the cortex showed evidence of injury with moderate to heavy radiation, its zones vary in response. The glomerular is usually described as less sensitive than the reticular or the fascicular zone (Engelstad; Holfelder and Peiper). The glomerular layer of the rabbit shows the least changes with severe cortical damage (Engelstad and Torgersen). Cottenot, Mulon and Zimmern described complete destruction of the reticular and fascicular zones after 54 Holzknecht units of medium quality roentgen rays had been administered in five treatments to each adrenal gland of 1 dog, and in changes in the glomerular layer suggesting compensatory proliferation. The examination was made quite

late post mortem. However, Rogers and Martin found the opposite to be true after the use of heavy unfiltered radiation; i. e., the fascicular zone was less affected than the other zones and maintained its histologic structure after being irradiated with 32 erythema doses, though other cells had disappeared. This zone alone was viable in the adrenal gland of 1 rabbit that died thirty-one days after 6.5 millicuries of unfiltered radium had been inserted in the organ (Lacassagne and Samssonow).

Although preservation of the medullary structure in the presence of clearcut degeneration or necrosis of the cortex has been described by several workers (Engelstad), severe fibrosis of the gland, except for small islands of normal cortical cells, was found twenty-six days after heavy radiation had been applied directly to the gland (Martin, Rogers and Fisher). Tsuzuki reported that degeneration of "medullary cells" occurred twenty-four hours after the administration of 32 per cent of an erythema dose while the cortical cells were little changed.

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## XII. EFFECTS ON BONE, CARTILAGE AND TEETH

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### BONE

In considering the histologic effects of radiation on bone it may be helpful to keep in mind certain facts regarding the structure of bone. Bone as a product of cell activity formed to give support and rigidity

is largely an intercellular substance of high mineral content. Minor alterations of the physical or the chemical structure of acellular tissue may produce little visible change. The difference between viable and nonviable bone may be only a slight variation in staining reaction and in the appearance of osteocytes unless disruptive forces, such as trauma or infection, intervene. For this reason effects of radiation on bone are less easily estimated. Moreover, the actual intensity of a given dose of radiation may be greater in bone than in other tissues because of the secondary radiations from the calcium.

The effect of radiation on growing, repairing and formed bone will be discussed as observed in experimental work on animals and as incidental to therapy.

*Data from Experiments on Animals.*—Retardation or cessation of growth of bone in young animals is a quite constant effect of radiation, provided the dose is above the threshold of tolerance. Although the reports in the literature often give seemingly inconsistent data, these may be reconciled in part by keeping in mind that variability is characteristic of all biologic reactions and that the reaction of living tissue is conditioned by its age.<sup>1</sup> The damaging doses of roentgen rays for bones of an infant and a child have been roughly estimated as 25 per cent and 50 per cent, respectively, of the erythema dose for an adult (Flaskamp). Difference in capacity for growth at different ages may explain variations in reaction when two bones of the same animal, as tibia and femur, are exposed simultaneously (Dahl; Engel). Generally, the degree of retardation is in direct relation to the dose up to the point of maximal response (2,400 to 3,000 r of high voltage rays, according to Gall and co-workers; 360 milligram hours, according to Engel), but the degree of retardation may be greater than the proportional increment of dose (Brooks and Hillstrom). Fractionation of the total dose and protraction of the interval between exposures greatly lessen the effect on growth (Bisgard and Hunt). As would be expected from observations on other tissues, equal doses of high voltage and supervoltage roentgen rays have been shown to produce identical effects except for slight difference in the timing of the reaction (Gall and co-workers).

Definite inhibition of growth<sup>2a</sup> has been observed in newborn and very young animals of different species,<sup>2b</sup> and this effect may be seen

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1. Baunach. Engel. Iselin. Scott.

2. (a) Moderate amounts of roentgen radiation (750 to 1,500 r at 200 kilovolts) given in a single dose through a portal 5 mm. in diameter over the distal femur to young rats produce slowing of the longitudinal and the transverse growth. The effect depends chiefly on the age of the animal. No changes in bone salts were brought about. (Hinkel, C. C.: *Am. J. Roentgenol.* **47**:439, 1942.) (b) Försterling. Perthes. Tribondeau and Récamier.

after as little as 18 per cent (Marie and co-workers) or 40 per cent (Brooks and Hillstrom) of an erythema dose given in a single treatment. After 2,600 r given in one exposure to young rabbits, growth was barely appreciable by roentgenograms for several weeks (Regen and Wilkins, 1936a). Brooks and Hillstrom, using rabbits, compared growth curves of normal bones with those of bones given a single exposure of 1 erythema dose and found an inhibitory effect in a change of rate but not of duration of growth. Complete cessation of growth was simultaneous in the irradiated and in the corresponding normal bone. The authors suggested that the destruction of cells with greater growth capacity and shorter life would diminish the rate without necessarily shortening the period of growth. Bisgard and Hunt, however, described a slower but constant rate of growth which stopped abruptly just before growth had been completed in the normal bone.

Bones of animals stunted by 600 r showed a decrease in phosphatase activity, most marked in the metaphysis, beginning a few days after exposure, reaching a minimum value within one to three weeks and recovering after four to five weeks (Wilkins and Regen, 1934a). This was not associated with change in the electrolytes (Wilkins and Regen, 1934b).

The final effect of radiation on developing bone depends on the number of cells damaged beyond recovery. The contour of irradiated growing bone is usually close to normal (Brooks and Hillstrom). Where there is deformity, it is due to two factors: muscle strain and greater injury to one part of the bone than to the rest.

No stimulation of growth of bone—i. e., in the gross sense of increase in size—was noted in young rabbits after very small doses, 2 to 5 per cent of an erythema dose (Brooks and Hillstrom), but Baunach, using 25 per cent of an erythema dose, thought there was a suggestion of greater growth.

The two histologic changes most frequently described in dwarfed bones of animals as an effect of radiation are early alteration of cartilage cells and disorientation of endochondral ossification. The earliest changes in epiphysial cartilage cells, such as swelling, pyknosis and loss of columnar pattern, were observed two weeks after 50 per cent of an erythema dose in rabbits (Bisgard and Hunt) and one to two weeks after 600 r (200 kilovolts) in rats (Gall and co-workers).

The outer primitive cartilage cells never show more than slight change, but there seems to be some inhibition of their development into chondroblasts (Gall and co-workers). The least differentiated cartilage cells next to this outermost layer of primitive "mother" cells are the first to be affected (Gall and co-workers). Increasing amounts of radiation affect still better differentiated cells, and the columnar pattern becomes quite disorganized. A simultaneous change in the matrix



occurs which has been described as a coarsening and later a loss of fibrils. The same effect was seen in the epiphysial cartilage of the right femur and the right tibia (although most marked in that of the femur) two weeks after a 5 mg. radium needle was implanted subcutaneously for three days on the lateral side of the proximal end of the right tibia (Engel). After 1 erythema dose given in a single exposure to rabbits, some cartilage cells were severely damaged and disappeared, while others remained normal (Brooks and Hillstrom). Irreparable arrest of the growth of cartilage was produced after 550 r to 880 r had been given in fractionated doses (Baunach). Focal necrosis of cartilage has been observed in animals irradiated with 1,650 r (fractionated) (Baunach).

Some degree of recovery of cartilage has been described even after severe damage, and occasionally a large portion has regained its normal state. Usually the proliferating cells have lost their polarity, and even though they have the appearance of normal cells, there is no effective growth (Gall and co-workers). Bisgard and Hunt described complete loss of pattern with extreme degenerative changes in cartilage cells some days after 1,540 r was administered in a single dose to long bones of rabbits 4 weeks old, but on the thirty-sixth day columnar structure and normal-appearing cartilage cells were again recognizable. The final result is irregularly thickened and often partly ossified cartilage.

The exact mechanism of the interference with endochondral ossification has not been clearly described. This seems to be due to difficulty in estimating the pathologic changes in osteoblasts. Dahl described suspension of vascular invasion and of ossification of the cartilage. Gall and co-workers, using rats, observed increased osteoblastic activity accompanying the earliest change in chondroblasts one to two weeks after exposure of bone to 600 r. Even though the osteoblasts appeared to have been injured, there was slight excess of bone deposit on the primary cartilaginous trabeculae. The ossification of these primary trabeculae increased with higher doses; they became extremely thick and, in the absence of the propelling force of the growing cartilage, formed a bony plate at the epiphysial line. The bone thus formed was abnormally brittle (Gall and co-workers).

On the other hand, Dahl described premature calcification and inhibition of endochondral and periosteal ossification soon after epidermicidal doses were directed to the adjoining ends of the tibia and the femur in rats 12 to 18 days old. Calcification appeared first in the metaphysis on the third day and in the epiphysis on the fourth day. In the femur the endochondral ossification of the metaphysis was completely suspended by the fourth day, but in the tibia it continued sluggishly. Similarly, periosteal ossification ceased almost immediately in the femur but only after four to eight days in the tibia. Endochondral

ossification and periosteal ossification are thus of the same radio-sensitivity (Dahl). Resorption of the diaphysis occurred in the tibia in the course of three to six weeks. The inhibition of ossification disturbed the normal balance of new bone formation and resorption. The continued resorption, calcification and devascularization of marrow and periosteum produced bone which was partly devitalized, partly sclerotic and heavily calcified (Dahl).

In summary, studies of the epiphyses of animals after the administration of roentgen rays have demonstrated high sensitivity of cartilage cells and less obvious sensitivity of osteoblasts. There is loss of polarity of cartilage cells and disorganization of endochondral ossification. Calcification is disturbed. The normal rhythm of bone formation and resorption is lost. Histologic evidence of injury is found in both chondroblasts and osteoblasts, and the intercellular matrix of each is affected.

Evidence regarding the effect of radiation on healing bone is likewise lacking in uniformity, but it is probable that this is due to difference of methods as well as to variation in interpretation. Cluzet, using moderate doses of low voltage roentgen rays, found that in rabbits and dogs there was marked delay in the formation of callus whether radiation was given before or after the bone was broken and even though the dose was insufficient to produce dermatitis. But Fukase has been cited to the effect that a dose of 400 r stimulated the formation of callus. Simon, using adult rabbits, transplanted segments of bone 1 cm. long from one bone to another and studied the effect of roentgen rays on absorption and union. Roentgenologic, gross and histologic observations indicated that the rays accelerated absorption of the transplant and also the process of union, which nevertheless followed normal lines. He expressed the belief that this was not due to stimulation of growth but was the result of rather greater injury to the transplant than to the adjacent tissues.

Brooks and Hillstrom made equal defects in the shafts of the right and left ulnas of young rabbits by subperiosteal resection and then submitted the right foreleg of each to an erythema dose either immediately after operation or on the third and sixteenth postoperative days. Although there was no perceptible difference in the amount of new bone formed or in the duration or the completeness of repair, the growth of the irradiated ulna was inhibited in each case.

Albee, using young adult to middle-aged rabbits, claimed no difference in the time or the completeness of union or in bone growth between irradiated and nonirradiated fractures or resected bone defects, even after massive doses had been given in several exposures five days apart.

On the other hand, Regen and Wilkins (1936b) found marked delay in the healing of fractures in ulnas of adult rabbits as compared with

the control. Animals were exposed to roentgen rays twice before fracture; the first dose was 1,112 r, and the second, two weeks later and one day before operation, was 622 r. Preliminary experiments had shown that phosphatase activity was consistently low in normal bones for four to five weeks after such treatment. The roentgen treatment given before fracture inhibited the rise of phosphatase activity which normally accompanies healing of bone. The callus was small in amount, and rigidity was delayed (Regen and Wilkins, 1936b).

Bade and Küntscher described moderate changes in bone cells of dogs after resection of a segment of radius and exposure to heavy radiation. Fusion of the cut ends of the radius did not occur as it did in the nonirradiated resected bones.

There are few experimental data which tell us what radiant energy does to adult normal bone. Nageotte suggested that although the gross appearance of dead bone may not be distinctive, degenerative changes in bone cells would be observed more frequently if looked for properly. On the other hand, Flaskamp expressed the opinion that healthy bone of adults is resistant to radiation.

Absorption and decalcification, with or without spontaneous fracture, have been seen in animals after exposure to heavy radiation,<sup>3</sup> implantation of radon seed (0.16 millicurie) (Franseen and co-workers) or as a result of chronic radium poisoning.<sup>4</sup>

Because of the tendency of heavy metals to be deposited in bone, radium poisoning has been especially productive of information in relation to the effect of radiant energy on bone.

Thomas and Bruner, studying chronic radium poisoning in rats, found both destruction of bone and increased growth. From 40 to 60 micrograms of radium chloride was given by subcutaneous injection during periods of one hundred and seventeen to one hundred and ninety-one days.

The elimination of the radium was found to be rapid. Approximately 35 per cent was excreted the first day of injection, and a lessening amount thereafter. Only 45 to 50 per cent of the radium was present in the body a week after injection, a large part of this remaining temporarily fixed in the tissues. At death, sixty to seventy-five days after the final injection, 24.6 per cent of the radium had been retained, and all but 1 per cent of this was fairly uniformly distributed throughout the skeleton, paralleling the deposits of calcium. The authors suggested that the rate of the elimination of radium serves as an index to the normal calcium turnover in the bones.

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3. Bade and Küntscher. Récamier and Tribondeau.

4. Sabin and others. Tribondeau and Récamier.

The destructive effect of the radium was described by Thomas and Bruner as follows: "The radium which is present in the bone disintegrates and expends much energy in the form of radiations. Ninety per cent of the energy set free resides in the swift alpha particle." Because of the high kinetic energy of the alpha particle, "it is capable of disrupting molecules, of producing ionization in gases, and of decomposing liquids into their constituent elements." "A rat containing only 15 micrograms of radium in its system absorbs over 2,000,000 alpha particles per second. The 25 ergs of energy so produced exercises a disturbing physiological effect upon the system of the animal. The greater portion of these radiations is concentrated upon the marrow and the immediate tissue. Not only do the bone cells receive the disruptive effect of most of the alpha rays but they also receive the shock of the recoil atoms. The combined effect might cause the destruction of cellular matter, which would be carried away in solution, leaving the bone brittle and fragile."

The most uniform change in the bones was thinning and partial decalcification of the central trabeculae, which resulted in pathologic fracture in at least 3 rats and increased fragility of the bones of all the animals. This was associated with concentration of calcium salts in the parts of the bones nearest the joints. In several animals there was necrosis of bone and also of soft tissue at the costochondral junctions. This was followed by proliferation of fibrous tissue and thickening of the periosteum and endosteum. Similar necrosis and replacement by fibrous tissue was seen in the sternum of 1 animal.

Rabbits that were poisoned and subsequently died from large doses of thorium dioxide showed in the main similar rarefaction of the femoral diaphysis. In addition, a preeminent role of the osteoclasts was suggested by the large numbers massed around the trabeculae (Lambin).

*Data from Studies of Man.*—Information on the effects of radiation on human bones is incidental to therapeutic irradiation of bones and to poisoning from radioactive substances. Changes resulting from the therapeutic application of radiation are most often seen in the rib, the femur, the mandible and the maxilla after long-continued exposures.

The possibility of stunting bones of children by therapeutic radiation to soft parts over epiphyses must be kept in mind (Walter). Bisgard and Hunt gave 2 illustrative cases. A child 1 year old had radium applied over a nevus on the dorsum of the right index finger. The finger failed to lengthen and at the age of 6, was shorter than the little finger. Roentgenograms showed that all the epiphysial lines of this finger except the proximal phalanx were closed. A child of 5 years had radium and roentgen irradiation of a tumor on the medial aspect of the knee. A varus deformity and shortening of the leg developed. When

this was presented for correction, ten years later, roentgenograms showed that the epiphysial junction on the mesial aspect, which had been nearest the source of radiation had closed but that the junction on the lateral side was open.

Spontaneous fracture resulting from radium poisoning or from therapeutic application of radiation does not usually heal normally; either fibrous union or pseudarthrosis results.<sup>5</sup>

Both absorptive and sclerotic processes, as well as spontaneous fracture, have been observed in the ribs, especially after treatment for carcinoma of the breast.<sup>6</sup>

How often spontaneous fracture of the neck of the femur is a result of injury from radiation during treatment of cancer of the pelvis is not entirely clear. The data are difficult to evaluate, since the normal disadvantages of the neck of the femur as an anatomic unit make it peculiarly liable to fracture from minor trauma. The fact that the incidence of fracture is higher among patients who have had radiation to the pelvis (2.1 per cent of 471 patients—Dalby and co-workers) than in the general population of the same age group is suggestive. In most of the cases the conditions are similar.<sup>7</sup> Fracture usually occurs months after irradiation of the region and is not associated with metastasis. The condition is sometimes bilateral. In cases reported by Dalby and co-workers the initial changes usually developed in the superior portion of the femoral head, close to the neck (Peck). Osteoporosis was often present, but in a case of subcapital fracture of the left hip the heads of both femurs remained dense in spite of atrophy of other bones around the joint (Batt and Hampton). Normal healing is infrequent, but fairly often there is fibrous union. Microscopic studies in 3 cases (Dalby and associates) showed similar changes: extreme obliterative endarteritis of nutrient vessels, "rarefying osteitis" with absorption and partial loss of calcium, and foreign body giant cell reaction about fragments of necrotic bone. Fibrous union occurred in one case, very dense callus in another and pseudarthrosis in the third. In 1 case the neck of the opposite femur showed marked osteoporosis and young fibroblastic proliferation with small islands of cartilage and a slight amount of new bone.

A pure radiation effect is infrequent in the maxilla and the mandible, but destructive lesions of these bones are commonly seen by radiologists as a result of the combined effect of radiation and infection after treatment of intraoral carcinoma. The effect of infection in enhancing damage to bone is well illustrated by the necrosis of the mandible in

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5. Flinn. Freid and Goldberg. Jacobsen.

6. Freid and Goldberg. Rose.

7. Bade and Küntscher. Batt and Hampton. Kropp. Philipp.

phosphorus poisoning. Regaud observed certain peculiarities of necrosis of bone following irradiation of carcinoma of the buccal mucosa:

1. Radionecrosis of bone may take place beneath intact skin or mucous membrane.
2. The macroscopic integrity of bone is maintained even after exposure to heavy radiation.
3. Infection or trauma added to radionecrosis produces marked gross disintegration of bone.
4. Sequestration in radionecrosis is much slower than in necrosis due to trauma or infection.
5. Bone killed by radiation is resistant to histolytic solvents.

From these facts Regaud drew the following conclusions: Bone is more vulnerable than surface epithelium. This vulnerability is probably inherent in the supporting substance and not in the bone cells. There is no elective sensitivity of normal adult bone to radiation but rather a diffuse radiosensitivity conditioned by calcification of the fundamental substance. The secondary rays act mainly on the lamellar substance, altering in some way the physical constitution of this substance without producing gross evidence of change as long as the bone remains free from trauma and infection. The macroscopic integrity of heavily irradiated bone is explained by the presence of an amorphous mineral substratum and by the extreme slowness of the normal changes in bone. An alteration in the ground substance is responsible for the greater susceptibility to infection and less susceptibility to histolytic solvents, which is also seen in all collagenous substances affected by radiation.

Ewing made most useful observations of irradiated human bones from the standpoint of histology. He described "productive osteitis" in 3 young adults who had received very heavy roentgen radiation or radium packs, or both, followed in shorter or longer time (three to nine months) by amputation. In case 3 a spontaneous fracture occurred four months after the use of radiation, and eighteen days later, at the time of amputation, there was no trace of callus or of inflammatory reaction. The shaft appeared devitalized and was hard, dense and "polished as ivory," and around it there was new subperiosteal bone, 1 to 3 mm. thick. This could be stripped off with ease, leaving a finely granular bony surface, and suggested an involucrum forming about a sequestrum in the process of absorption.

. . . In the portions of hard bone taken from the shaft away from the tumor, the cell spaces appear to be larger and more irregular, the canaliculi are larger, widened, very irregular, reduced in numbers, and the communications between

adjoining cells are very imperfect. Numerous abnormal spaces appear between the lamellae. The lamellar substance appears very hyaline and brittle. . . . It is admissible to conclude that these changes signify severe degeneration of the bone cells, destruction of many cell processes and closure of many canaliculi and changes of undetermined nature in the lamellar substance.

In the other 2 cases there was an extreme degree of radiation dermatitis together with an impairment of circulation due to vascular changes. In case 1 the bone as a whole did not appear devitalized although some necrosis, decalcification and absorption had occurred, but in case 2 the bone was either necrotic or devitalized and osteoblasts were not seen over a large area. A striking feature of all 3 cases was the brittleness and fragility of the greatly thickened and dense bone. Endosteal bone formation was especially conspicuous.

It has been estimated that poisoning<sup>8</sup> occurs in only 2 per cent of the several thousands of persons exposed in various occupations and in laboratories where radioactive compounds are handled (Ewing).

Regardless of the form in which radioactive material is taken into the system, whether gaseous or solid, the greater part is excreted within a short time, the rapidity of elimination depending partly on the avenue of entry.<sup>9</sup> That which remains is stored in the reticuloendothelial cells. The greatest concentration has been found in the lungs, the spleen and the bones, including the bone matrix (Barker). The rate of elimination varies in different subjects and may continue for many years after exposure (Flinn; Schlundt and Failla). The occurrence of toxic change and the degree of poisoning depend to some extent on the ability of the organism to eliminate radioactive substances and possibly on a certain individual susceptibility. Barker examined the tissues of a person dead of radium poisoning and found the radioactive material hardly sufficient to produce the systemic changes resulting in death.

Pathologic aspects of radium poisoning in man have been reported in considerable detail by Martland, who studied the effect of occupational

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8. Rajewsky stated that the "lower limit of the dose which will effect a definite change in the metabolism is about  $1.5 \times 10^{-5}$  curie-hours per cubic centimeter. . . . This dose exceeds the normal radium content of the tissues by only about 60 to 90 times and is hence considerably less than the 'tolerance dose' in roentgen or radium therapy." Extremely small amounts of radioactive substance deposited in tissue may produce serious damage. Rajewsky stated that 1 microgram of radium equivalent may produce deadly injury to the organism. To provide this residuum, with the normal rate of elimination, it would be necessary to introduce 100 micrograms orally or 20 micrograms intravenously or by inhalation. The tolerance dose of radium for human tissues is appreciably lower than that of roentgen rays as measured by ionization chambers. This is due chiefly to the great biologic effectiveness of the alpha particles of radium.

9. Barker. Barker and Schlundt. Seil, Viol and Gordon.

exposure to radioactive salts. The workers used a dial paint composed of insoluble sulfates, rendered luminous by small amounts of mesothorium and less radium and radiothorium. The injurious effects were due almost entirely to the disintegration of these substances to form alpha particles. The preponderance of mesothorium in the paint made it particularly toxic, owing to the fact that greater numbers of alpha particles are formed from the disintegration of this substance than from that of either radium or radiothorium (Martland).

At the time of reporting, Martland stated, 18 deaths had been attributed to radium poisoning in the group of exposed persons studied. These deaths occurred three to eight or more years after cessation of exposure. The radioactive substances were confined to the bones, the distribution being fairly uniform throughout the skeleton, with the highest concentration observed in the most heavily calcified portions, as the dense outer cortex. But uniformity of distribution of radium compounds in bone as a usual thing has been questioned by Schlundt and Failla.

The changes in the bone induced by radium are described by Martland as a healing osteitis comparable to the nonsuppurative osteomyelitis of Garré except for the more intense inflammation of the former. Non-cellular myxomatous fibrous tissue with many hyperchromatic dividing fibroblasts fills the marrow, which later becomes dense, acellular and ossified. Bone may become nonviable, but necrosis is not usually present unless there is superadded infection, as there commonly is in the jaw (Hoffman, 1925). Unlike phosphorus poisoning, which is the only condition in which an equal degree of necrosis of bone is seen, there is neither involucrum nor healing (Ewing). Osteogenic sarcoma developed in 5 of the 18 persons dying of radium effect reported by Martland. The tumors appeared five to eight years after the contact with radium had ceased. In 2 instances sarcoma was present in two different bones.

Bazy and Coste reported lesions in bones several years after treatment with 4,400 micrograms of thorium dioxide in divided doses. They considered that the lesions resembled those described by Martland in radium poisoning.

*Production of Tumors.*—Malignant change as a result of exposure to radiant energy has been described somewhat less frequently in bone than in other tissues. Nearly all of the recorded tumors produced in bones of animals have been diagnosed as osteogenic sarcoma, but among the reported tumors 1 was diagnosed as chondrosarcoma (Ludin, 1934), 1 as fibrosarcoma (Daels and Biltris, 1931) and 1 as Ewing's tumor (Schürch and Uehlinger, 1937). Tumors have been described in chickens, rats, mice and a guinea pig as well as in the more commonly used rabbit.



*Tumors of Bone Produced Experimentally with Radiant Energy*

Author	Type of Radiation	Filtration	Time of Radiation	Time from First Exposure to Radiation to Appearance of Tumor or Death	Animal	Type of Tumor
Dael, F., and Biltris, R., 1931 *	Radium bromide, amount minute but n.s.	.....	.....	2 years and 1 month	Guinea pig	Fibrosarcoma
Schürch, O., and Uehlinger, E., 1931.....	Radium, 1 mg.	Platinum	20 days	1 year and 6 months	Rabbit	Osteogenic sarcoma
Sabin, F. R.; Doan, C. A., and Forkner, D. E., 1932	Radium chloride in solution, 61.2 micrograms	.....	1 year and 5 months	1 year and 5 months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1935.....	Radium, 2.5 micrograms, 3 animals	.....	.....	1 year and 9+ months	Rabbit	Osteogenic sarcoma
Ross, J. M., 1936.....	Radium, 1 mg.	.....	.....	1 year and 11 months	Rabbit	Osteogenic sarcoma
Hollner, H., 1937.....	Radium, 2,480 milligram hours	.....	Divided doses over 2 years and 3 months	2 years and 3 months	Rabbit	Osteogenic sarcoma
Dael, F., and Biltris, R., 1937.....	Radium sulfate, 0.006 mg.	.....	.....	4 years and 10 months	Chicken	Osteogenic sarcoma
Dael, F., and Biltris, R., 1937.....	Radium sulfate, 0.003 mg.	.....	.....	4 years and 11 months	Chicken	Osteogenic sarcoma
Sabin, F. R.; Doan, C. A., and Forkner, D. E., 1932.	Mesothorium, 30.6 micrograms	.....	11 months	11 months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1935.....	Mesothorium, 2.5 micrograms, 2 animals	.....	.....	1 year and 9+ months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1937.....	Mesothorium, 5 micrograms	.....	.....	3 years	Rabbit	Ewing's tumor
Selbie, F. R., 1938.....	Colloidal thorium dioxide (thorotrast), 0.2 cc.	.....	.....	1 year and 5 months	Mouse	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937.....	Radon tube, 0.69 milliecurie	Glass	.....	1 year and 3 months	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937.....	Radon tube, 0.83 milliecurie	Glass	.....	1 year	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937.....	Radon tube, 1.08 millieuries	Glass	.....	1 year and 1 month	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Vincent, R., 1929.....	Röntgen rays, 160 kilovolts, 2,000 r in 2 equal exposures, and streptobacillus abscess	.....	6 months	11 months	Rabbit	Osteogenic sarcoma
Lacassagne, A., 1933 *	Röntgen rays, 160 kilovolts, 1,980 r in 3 exposures, and injection of streptobacilli	.....	5 months	5 years and 2 months	Rabbit	Fibrosarcoma
Ludin, M., 1934 †.....	Röntgen rays, 120 kilovolts, 8,000 r in 40 equal doses	.....	6½ months	6½ months	Rabbit	Chondrosarcoma

\* The tumor probably originated from the periosteum, but this is not entirely clear.

† In 1930 Ludin published a short notice of chondrosarcoma arising in the tibia of a guinea pig after a long period of repeated treatments with radiation. Except for the fact that the animal was a guinea pig instead of a rabbit and that details of the experiment were not given, the report was identical with the one included in this table.

Note: Several writers have ascribed to Marie, Clunet and Raulot-Lapointe the first report of osteogenic sarcoma produced in an animal by the use of radiant energy, but we could not find any such report.

As may be seen from the accompanying table of experimentally produced tumors, the factors involved are not easily evaluated. Tumors have followed relatively short as well as long periods of exposure to small and larger amounts of gamma, beta and roentgen rays. Time is an important element, but whether any responsibility for the tumors can be assigned to severe necrosis of bone or chronic inflammation is not clear. Certain of the tumors diagnosed as osteogenic sarcoma reported by Martland in radium dial painters (27 per cent of the deaths resulted from osteogenic sarcoma) arose in the intensely inflamed tissue of radiation osteitis. Several authors have described cancers and benign tumors developing in bones which were the site of tuberculous processes which had been treated by roentgen rays (Selbie). However, bone tumors secondary to inflammation alone are unknown in man, the only recognized predisposing factor being Paget's disease.

*Summary.*—The reaction of formed bone to radiation is primarily a lowering of vitality which in some instances progresses to necrosis. This may occur without change in the bone's structure or general appearance, the only indication of the injured state being a slight alteration of the osteocytes. Usually injury is indicated by excessive resorption or by overgrowth of bone as if an attempt at repair were being made, and the synchronism of resorption and new bone formation is lost. Atrophy and eburnation of bone are recognized late radiation changes. Irradiated bone, because of the impairment of its vitality, is peculiarly sensitive to infection and trauma and these are in large part responsible for necrosis and sclerosis.

There is little evidence pointing to the reactions of osteocytes, osteoblasts and osteoclasts. On the other hand, the changes in chondroblasts, so carefully studied, demonstrate marked sensitivity of these cells. An equal interest in the gross and the microscopic changes following irradiation of formed bone is much needed.

The matrix of bone is affected, and although the nature of the change is not clearly understood, it may be recognized by certain characteristics, such as (*a*) increased brittleness and (*b*) absence of sequestration.

Adult bone is probably more sensitive to radiant energy than skin or mucous membrane. Although it has received less attention than epiphysial cartilage, there is evidence pointing to like sensitivity.

#### CARTILAGE

Hyaline cartilage as contrasted with epiphysial cartilage is relatively insensitive to radiation (Dahl) but not to such a degree that its reaction can be neglected when one is considering irradiation of the ear, the nose or the larynx, for example. Perichondritis in these tissues after their

irradiation may be a serious complication, often not fully appreciated at first because of the long period, sometimes several years, before it develops (van Rossem; Schmidt). Changes in the costal cartilages following irradiation of these structures are of no practical importance and are seldom described. Rose, however, mentioned "softening" of cartilage, as well as fibrosis of muscle and of lung and atrophy of bone, some time after heavy radiation had been applied in multiple divided doses for carcinoma of the breast. Many clinical observations of radiation chondritis and perichondritis are available, but the pathologic aspects have been slighted. Indeed, the injury to cartilage has been considered, not altogether erroneously, as due to damage of the blood vessels (Schmidt).

Thies described radiation changes in the ensiform cartilage of the guinea pig. His description is of interest in showing a sequence of cell reactions, various stages of which are familiar to the pathologist. Twenty-four hours after a six hour exposure to 20 mg. of radium bromide the nuclei of the perichondrium and of the superficial cartilage showed various stages of degeneration with accompanying vascular engorgement and infiltration by polymorphonuclears, lymphocytes and a few eosinophils. Six days after irradiation the superficial layer of cartilage appeared broadened and the cells appeared hypertrophied. The perichondrium was markedly thickened and hyperemic. Two weeks later multinucleated cells were present in the superficial cartilage. The matrix was not altered.

Like bone, adult cartilage stands large amounts of radiation without marked gross change so long as it is protected. When it is infected or traumatized, extensive necrosis and sequestration may occur.

#### TEETH

Experimental studies have emphasized the effect of radiation on developing teeth. Tribondeau and Récamier exposed the anterior lateral aspects of a kitten's face to roentgen rays for ten minutes each six times in a period of two weeks and noted retardation in the development of the teeth, which appeared more marked than that in the development of the bones of the face. Leist and Smith have made similar but more detailed observations.

Leist described changes in the teeth of rats and young dogs exposed to doses for the most part greater than those used for therapy. Eight rats were treated. The radiation was given to the anterior part of the head (120 kilovolts; 28 cm. focal skin distance). The treatment varied: One rat received only 1.5 Holzknecht units; 5 rats, 15 Holzknecht units unfiltered; 2 rats, five and six exposures to 1.5 Holzknecht units each

and later three exposures to 15 Holzknecht units each. Changes were seen only in the growing gnawing teeth. In each case, the odontoblasts were affected and the formation of dentin interrupted, but the enamel and the cement remained unchanged. The injury to the odontoblasts of the germinal layer was more or less severe. Masses of degenerated odontoblasts were found in niches formed in the dentin as a result of the inhibitory effect of the rays. The arrest of growth resulted from the destruction of the odontoblasts.

A similar result was produced by irradiating 3 puppies still with their milk teeth. The jaw alone was irradiated in 3 puppies, in 2 with roentgen rays and in 1 with radium. The roentgen doses varied from 8 to 15 Holzknecht units in a single exposure. One puppy received four treatments, two with filtered and two with unfiltered roentgen rays, two to four weeks apart; the other was given two exposures to unfiltered and one exposure to filtered rays. The third puppy was treated with 100 mg. of radium (in a glass tube enclosed in a silver tube with an outer capsule of platinum 0.3 mm. thick and gutta percha) three times at intervals of four weeks; for one treatment an additional zinc filter was used. The observations were delayed dentition, slowed rate of growth and incomplete development. The odontoblasts were few and much altered in shape, with their nuclei shriveled and disoriented. The pulp showed some early atrophy. Mature teeth were similarly affected but only after 2 to 5 erythema doses. There was also no tendency toward regeneration as in the case of undeveloped teeth.

The amount of radiation determined whether growth was partly or completely stopped, as well as the degree of regeneration. Loss of teeth was in great part due to injury of the peridental membrane and of the alveolar process itself.

Smith attempted to determine the effect on growing teeth of smaller exposures to radiation. He exposed 5 rats of the same litter, 6 days old, to radiation that was thought "to be equivalent to the individual exposure or repetitions of exposures to which a child might be subjected in securing dental roentgenograms." Exposures were given over a three week period (focal skin distance, 7 inches [7.78 cm.]; 10 milliamperes; spark gap, 3 inches [7.62 cm.]). The time of exposure varied from five to twenty seconds, and from three to six exposures were given weekly. One rat of the same litter served as control. The teeth were examined histologically ten days after the last exposure. Very careful transverse measurements and serial sections of jaws and teeth revealed no structural defects, but the method of examination would not bring out diminution of length. The author explained the difference in his results as compared with those of Leist by the amounts of radiation given in individual doses. Although the cumulative radiation in Smith's

cases equaled some of the individual doses found to be injurious by Leist, the individual doses Smith used were not comparable.

As in the case of phosphorus poisoning, the jaws and the teeth frequently show evidence of injury from radiation insufficient to be manifested by injury elsewhere (Leake).

In minor degrees of radiation injury, sclerosis and atrophy of the alveolus (Leake) have been described, as have fibrosis, "loosening of Sharpey's fibers" (Leist) and loss of vascularity (Tribondeau and Récamier). All these changes indirectly affect the teeth.

The direct effect of radiation on normal teeth has been less clearly understood. In 1926 Flinn stated that "so far as is known, radioactive material has no specific action" on teeth, although dental caries is certainly associated with radium poisoning (Martland). Del Regato stated the problems clearly in his description of changes noted in the teeth of a large number of patients who had been treated for oral or pharyngeal tumors by roentgen rays or radium. He said that it is exceptional for teeth to remain in good condition after exposure to heavy radiation even though they have not been within the field of the direct rays. The evidence of effect is partly subjective, many patients suffering only from hypersensitivity to stimuli during treatment. This sensitivity may disappear at completion of the treatment, but in some cases it reappears several months later, together with obvious dental lesions. The lesions, sluggish in development, as many radiation effects are, progress over a period of years and are generally, although not always, painless. In unusual cases there is complete destruction of teeth within twelve to eighteen months, and this may be very painful. The most common type of lesion is decay of the neck of the teeth, usually on the labial surface of the incisors and canines. The distinctive feature of the decay is its superficial character; it spreads over the surface of the neck before extending deeply and severing the root from the crown, both of which may be sound. In the case of the molars a more general spread of decay over the whole tooth is to be expected.

These lesions are not constant. Del Regato observed that the more severe lesions are seen in patients in whom there has developed intense radioepithelitis of the mucosa of the pharynx after a dose which does not ordinarily produce such a reaction.

*Summary.*—The direct effect of radiation on mature teeth has not been adequately studied. Such direct injuries as have been described are destruction of odontoblasts, interruption of dentin formation and atrophy of pulp. In growing teeth these injuries inhibit partly or entirely the dental development, with delay or failure of eruption.

Indirect effects, including loss of the teeth, are chiefly produced by injury to the peridental membrane and of the alveolar process.

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### XIII. EFFECTS ON THE SKIN

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The first deleterious effect noted from either roentgen rays or radium was that on the skin and its appendages.<sup>1</sup> By 1902 a series of 167 accidental roentgen ray burns was reported (Codman). The limiting factor in the treatment of many neoplastic processes is the tolerance to radiation of the adjacent or intervening skin. Consequently, more attention has been focused on the effect on this tissue than on any

1. Halkin. Marcuse. Unna.

other in the body. As many reviews of the data are available,<sup>2</sup> only such representative reports as permit giving a general picture of the changes occurring will be selected for consideration here.

The production of visible change in the skin (erythema) has long been used as the biologic standard of therapeutic radiation (Holthusen and Braun). This depends on several variables: the amount of radiation delivered, the amount of radiation absorbed (largely determined by wavelength), the time of exposure (protraction of interval between doses or fractionation of dose greatly decreasing the biologic effectiveness) and the size of the field (the larger field being the more effective because of the amount of back scatter produced). To establish a base for the erythema dose, the following figures from Ellinger are of value: Erythema will be produced in a 6 by 8 cm. field with 400 r of unfiltered soft roentgen radiation, 525 r of medium radiation and 600 r of hard radiation. Hudson, from studies of a number of x-ray apparatus installed in New England, found that with roentgen rays as ordinarily used an erythema may be produced with a dose of approximately 300 r at 100 kilovolts, and with a dose of approximately 700 r at 200 kilovolts. At higher voltages, such as 1,000 kilovolts, no satisfactory method of measuring doses in roentgens has been evolved.

However, with supervoltage the cutaneous reaction ceases to be the limiting factor in radiation therapy. An excellent discussion of the cutaneous reaction to radiation generated at 1,000 kilovolts is given by Stone and Robinson. They expressed the belief that there is no qualitative difference from the reaction obtained at lower voltages and that the assumed variations are due to variations in statement of the dose given. An erythematous reaction similar to that seen following roentgen ray therapy has been produced by a stream of alpha particles (Larkin). Histologically, there is little essential difference in the reactions produced by different types of radiation (Miescher, 1938).

There are two phases of the erythema. The first develops within a few hours to three days after the exposure to radiation and disappears after a day or two. From ten to twenty-eight days later the reddening of the skin again appears and is usually, as it fades, followed by the development of some degree of permanent pigmentation. Pigmentation does not, as a rule, develop after the initial erythema, but only when the more marked, later erythema has occurred. Miescher in 1925 expressed the belief that there are three phases, the maximum histologic changes occurring in the third phase, developing in the sixth week.

The early effects are caused by direct injury of those cells and structures which have been exposed to the radiation. The later effects

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2. Desjardins. Flaskamp. McKee. Wetterer.



are brought about by changes in the vascular bed of the corium and in the intercellular tissue and to some extent by changes in the epithelial cells themselves.

The role of the supporting tissues is well shown in the case of the rabbit's ear by transplantation experiments (Ungar and Warren). When a portion of an ear of a rabbit was heavily irradiated (120 milliecurie hours of radon in twenty minutes), that epithelium grafted to nonirradiated corium "took" in a number of instances, whereas normal epithelium transplanted to the irradiated corium failed to take.

The early injuries may be shown by chemical as well as histologic change. Thus rabbit skin irradiated with 220 to 1,100 r shows no visible change, but when ashed it shows salts increasing to reach a peak about thirty hours after exposure (Meltzer and Kühtz). There is slight evidence that the cholesterol and phospholipid contents of the stratum corneum are reduced by 900 r, unfiltered, generated at 120 kilovolts (Wile and co-workers). As the acute reaction develops and tissue disintegrates, the skin becomes more acid (Magath), the potassium falls and the calcium rises (Lieber) as does the nonprotein nitrogen (Urbach and Schnitzler).

Several clinical varieties of roentgen or radium injury to the skin are recognized. These are essentially varying manifestations of different intensities of the same fundamental changes.

When the initial erythema has subsided, there is apparently a period of latent injury, which probably represents a period during which the damage to the supporting tissues and to the epithelial cells themselves is gradually developing (Burrows and associates). For ten to twelve days after exposure capillary constriction and dilatation occur to varying degrees but dilatation becomes persistent from about the twelfth to the sixtieth day afterward (Ellinger).

Acute roentgen dermatitis appears as the initial area of erythema begins to fade and to show some slight brownish pigmentation. The skin turns deep red and becomes edematous. Following moderately heavy radiation, this erythema may be accompanied by marked edema and may result in desquamation of the epithelial cells (exudative radio-epidermitis) to a considerable degree and deposition of fibrin, sometimes accompanied by hemorrhage. This appears at the end of about four weeks of protracted external application of radiation. After about ten days the denuded area has again been covered with epidermis. A lesser degree of reaction is evidenced by scaling of the superficial layers, with bronzing or erythema of the underlying skin (desquamative epidermitis—Cutler and Buschke). These changes correspond to the "epithelitis" of mucous membranes described by Coutard.

Epilation usually occurs.

Sometimes the acute reaction is of the bullous type with large raised blebs containing abundant serum not infrequently becoming secondarily infected. If the radiation has been very heavy, ulcers, sharply defined and punched out, may appear about two months after the exposure. The development of acute ulcers is sometimes precipitated by secondary infection of regions in the irradiated field.

After the erythematous reaction, if the usual therapeutic dose has not been exceeded, there is ordinarily a fair return of the skin to normal, with the exception of some pigmentation due to local increase in melanin. Rarely hemosiderin is present, but it is not an important factor. Ellinger stated that pigmentation induced by roentgen or radium radiation does not migrate to the stratum corneum as does pigmentation caused by light, but rather is restricted to the basal cells of the epidermis and to the chromatophores. When one considers that frequently, following exposure to radiation, the thickness of the stratum corneum may be considerably reduced, the significance of this observation is open to question.

A variable that must be considered is that of susceptibility, individual and specific. Not only do skins of different animal species (Danysz) and of human beings have different thresholds of reaction, but also animals of the same species having different colors will react differently (Rudis-Jicinsky). In general, the heavier the natural pigmentation, the more radiation is required to produce cutaneous injury, as was first pointed out by Rudis-Jicinsky. Danysz showed early that the skin of the guinea pig is more sensitive than that of the rabbit. Even in the same animal parts of the body may vary in susceptibility. Thus, in the human being the exposed skin of extensor surfaces is more resistant than that of protected regions, such as the inner surface of the thigh. The scalp is one of the less sensitive regions (MacKee).

This variation in human susceptibility to injury explains in part the damage done to skin by roentgen ray treatment as administered by operators of beauty shops, through their ignorance (Hazen). It also partly explains why one radiologist may acquire a roentgen ulcer and carcinoma, while another with comparable exposure shows little change in his skin.

The stratum corneum is the portion of the skin most resistant to radiation, largely because its metabolism is relatively inactive and because the keratin is transparent to radiation. On the other hand, the stratum germinativum and the basal layer are relatively sensitive, the hair follicles are very sensitive, and their cells show cessation of mitotic activity and vacuolation before the shaft is lost.

In man epilation may be produced temporarily in about three weeks with about 400 to 500 r at 200 kilovolts and permanently with 700 r or more. The laboratory animals are more resistant than man, as may be seen from the accompanying table.

When animals are temporarily epilated, the hair may grow back white in regions where previously it was pigmented (Hance and Murphy). My associates and I have observed at the periphery of heavily irradiated regions of rabbits' skin a fringe of white hair several millimeters longer than normal.

During the first few days after a dose of radiation producing permanent epilation, the hair follicles show vacuolation, cessation of mitosis and focal necrosis. This is sometimes associated with slight polymorphonuclear and mononuclear infiltration but is generally without leukocytic response. During the first month the arrectores pilorum muscles show some increase in size due to vacuolar degeneration (Wolbach), followed by increasing atrophy, leading ultimately in many instances to complete disappearance.

*Doses Producing Permanent Epilation (Adapted from Ellinger [page 32])*

Animal	Dose	Type of Radiation and Filtration
Rabbit.....	2,500 r	200 kv., 2 mm. Cu + 1 mm. Al
Rabbit.....	2,000 r	100 kv., 2 mm. Cu
Dog.....	2,400 r	120 kv., 1 mm. Al
Mouse.....	1,900 r	Hard

The sweat glands are normally as sensitive as are the hair follicles (Flaskamp). The same dose that produces temporary epilation will produce temporary decrease in, or complete disappearance of, the secretion of sweat. Examined microscopically in the first few days, the epithelium shows prominently vacuolar degeneration, which sometimes leads to an unusual degree of distinctness of the so-called myoepithelial cells. About one month after exposure to radiation the basement membrane is doubled or even quadrupled in thickness, the lumens of the glands have virtually disappeared, and the connective tissue adjacent to the coils is increased in amount and hyalinized. Later the sweat glands may disappear entirely.

The sebaceous glands are likewise highly sensitive, and the reduction of their secretion begins within five or six days after exposure (Ellinger). Usually one month after a dose of radiation causing permanent epilation few sebaceous glands will be visible, and those persisting will frequently show some degree of peripheral keratinization, as well as accentuation of the basement membrane. The sebaceous glands tend to persist longer than the hair follicles, but not quite so long as

the coil glands. The dryness and the tendency toward scaling of irradiated skin are largely due to the disturbed function of the coil and sebaceous glands.

The immediate changes in the vascular endothelium are swelling and necrosis. They may be more marked in the endothelium of the capillaries than in that of the lymphatics. These endothelial changes may or may not progress to necrosis and thrombosis (Linser). If endothelial necrosis does not occur, but the damage has been severe, endothelial proliferation with various degrees of occlusion of the lumen may follow. The discussion of the evolution of the vascular lesions has been taken up in chapter VI and will not be repeated here. However, the fundamental importance of these lesions in the development of cutaneous damage must be kept in mind. If the radiation has been sufficiently heavy to produce changes in the vascular bed and in the connective tissue of the corium, this may in turn lead to the development of marked changes in the overlying epithelium. These late changes are seen in their most striking form in the chronic roentgen dermatitis produced by repeated small exposures to radiant energy.

This is most commonly seen on the hands as a result of occupational exposure.<sup>3</sup> The epithelium, which is disturbed in structure as well as in appearance of individual cells, may be hypertrophic or atrophic (Porter). In the hypertrophic form there is marked keratinization of the lesions; also, associated hyperkeratosis of varying size. The epidermis in general is thickened, the hair absent or sparse, the cutaneous folds exaggerated. The nails are brittle, distorted and cracked. This lesion may go on to the development of carcinoma.<sup>4</sup> In the atrophic form the skin is shiny, thin and scaly, revealing beneath it a web of telangiectasis. In such a region a roentgen ulcer may be produced by even minor trauma or infection. It is characterized by sharp edges and a clean base, and is intensely painful. In the reparative epithelium at the margin of such an ulcer, carcinoma may develop.

Roentgen carcinoma may be epidermoid, the more usual type, or it may be of the basal cell type. However, fibrosarcoma may originate rarely from the abnormal fibroblasts of the irradiated corium. Among 135 cases of radiodermatitis in physicians seen at the Mayo Clinic there were 39 in which carcinoma had developed (Leddy and Rigos). Twenty-eight cancers caused by radiation were reported by Witwer and Leucutia, and among these 8 had developed in doctors or dentists.

As far as the connective tissue is concerned, in the early stages of the reaction edema dominates the picture. When radiation effect has been sufficiently severe, there may be deposition of fibrin and necrosis

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3. Leddy and Rigos. Saunders and Montgomery.

4. Porter. Wile and others.

of fibroblasts, sometimes accompanied by slight leukocytic infiltration. This occasional necrosis and focal deposition of fibrin may appear even years after the primary radiation injury as a result of abnormalities induced in the fibroblasts when the skin was first irradiated (Wolbach). The collagen fibrils, which at first are only swollen, later tend to coalesce. The hyaline material thus formed is sometimes faintly basophilic and sometimes strikingly acidophilic in staining reaction and is very homogeneous. Among the denser collagen strands there may be some growth of young fibrocytes. Eventually the dense hyalinized collagen makes even more compact a mass than that seen in keloid. If there is appreciable damage of the skin, the basement membrane disappears from below the basal cells about three weeks after exposure to radiation and rarely, if ever, reforms.

Within the first few days after irradiation of skin the elastic fibers are swollen and more readily stained. Later their degeneration may be complete with heavier doses, and their loss is responsible for much of the atrophy and loss of normal contour in the irradiated cutis.

#### SUMMARY

Erythema is the first clinical evidence of irradiation of the skin. The cutaneous effect depends on alterations produced in the epidermis, the cutaneous appendages, the corium and the vessels. Following erythema, epilation and even ulceration may occur. Pigmentation is usual after doses producing a distinct erythema. Vascular thrombosis or occlusion by endothelial proliferation may damage the blood supply.

In a late radiation reaction, varying degrees of atrophy of the epithelium, hyperkeratosis and scaling may be clearly apparent. The epithelium becomes increasingly thin, with almost complete obliteration of the rete pegs and disappearance of the basement membrane. A considerable degree of distortion of the normal structure may occur, sometimes with the development of abnormal mitotic figures. The cutaneous appendages have partly atrophied or have disappeared. The fibrocytes may show distortion or gigantism. The collagen is dense and hyalinized, without elastic fibers. The vessels are telangiectatic or occluded. Neoplasia is a not infrequent late complication.

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#### XIV. EFFECTS ON STRIATED MUSCLE

Owing to its high degree of differentiation, striated muscle is resistant to radiation.<sup>1</sup> Even in zones immediately adjacent to complete necrosis due to implantation of unfiltered or lightly filtered radon needles, cross striations are still visible, and the transition from necrotic to apparently normal fibers is abrupt (Lacassagne). In fields where heavy external radiation has produced fibrosis and vascular damage, atrophy and other changes in the muscle fibers may develop as a secondary change. However, some atrophic changes are a direct effect of the radiation (Dobrovolskaia-Zavadskaia).

The experiments of Lacassagne are well planned to show the effect of interstitial radiation. Platinum needles or bare tubes containing

1. Danysz. Englmann. Lacassagne.

radon in amounts of from 5 to 55 millicuries were introduced into the lumbar muscle mass in rabbits. After forty hours there appeared a sharply defined zone of chalky white necrosis, surrounded by a zone of congestion with dilated capillaries and some hemorrhage. This inner zone showed loss of striations, fragmentation of fibers and homogeneity or massing of sarcoplasm. Peripherally, the muscle nuclei appeared normal in spite of these cytoplasmic changes, but farther in the nuclei had been broken in to a dust of chromatin. The amount of radon directly affected the size of the lesions, 5 millicuries producing a zone of necrosis 4 mm. in diameter and 55 millicuries producing a zone 15 mm. in diameter. The necrosis was produced rapidly, and prolonging the time the tubes were left in place changed the picture but little. Moreover, the extent of necrosis was fairly constant regardless of filtration (a radius of about 7 mm. from the tubes).

These experiments were extended by Dobrovolskaia-Zavadskaia, who studied especially cytologic changes in the muscle fibers. She found that the alterations in muscle occurred in more or less concentric zones about a focus of interstitial radiation. (She used from 2 to 14 mg. of radium, left in place from fifteen days to nine and one half months.) The central zone of coagulation necrosis she ascribed to beta radiation; about this she found a zone of atrophy due to gamma radiation. The atrophic changes are not pathognomonic, but resemble those seen in typhoid fever, scarlet fever and some intoxications. The fibers are shrunk, but the nuclei increase in number and tend to cluster together. They persist even when practically all cytoplasm has disappeared. This increase in nuclei (Haendly) and their migration into the sarcoplasm are not unlike what is seen in attempted regeneration of muscle injured by other means. Swelling and rounding of nuclei are not infrequent (Dobrovolskaia-Zavadskaia; Fenn and Latchford). Mitosis is not seen. Karyolysis occurs as a result of treatment with heavy radiation.

In most instances of roentgen therapy, only the atrophic changes appear. Damage to the sarcoplasm, particularly to its contractile elements, is striking. With light radiation there is progressive diminution in volume without striking changes otherwise, whereas, with heavy radiation there is formation of vacuoles, which distort the myofibrils. These vacuoles are usually hydropic but may be fatty (Okada). Sometimes Zenker's degeneration appears. Usually the cross striations are diminished in number or disappear early (Lazarus-Barlow). At the periphery of the zone of atrophy there is frequently a striking contrast between adjacent fibers, some being markedly shrunk, others being virtually unchanged from normal. Occasionally at the periphery of the zone of atrophy there may be even some hypertrophic fibers. With

the disappearance of muscle fibers there is a striking degree of fibrosis (Thies) in which the collagen may become more or less hyalinized. In some of the less damaged fibers there is a striking waviness of the myofibrils that gives them a ribbon-like appearance. In such fibers the longitudinal fibrillation tends to be exaggerated and the cross striations to be somewhat diminished.

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#### XV. EFFECTS ON THE GERM PLASM AND THE EMBRYO

##### THE GERM PLASMA

The effects of radiation on the germ plasm were recognized early and have been utilized extensively by experimenters both in botanic and zoologic fields to produce mutations (Muller). Radiation has sometimes caused changes of form of varied severity in those offspring that are viable and has produced effects leading to premature death in others.<sup>1</sup> Since irradiation of the ovaries usually is sufficiently intense to interfere with subsequent development of the ova, this degree of sterility is one of the factors explaining why such radiation is not more often followed by unhealthy or maldeveloped offspring (De Rényi and co-workers).

The studies of Bagg (1929) and Bagg and Little have been of considerable interest. Some of their mice descended from roentgen irradiated parents were followed to the ninth generation (Bagg, 1929). The inheritance of abnormal development was interpreted as mendelian and also recessive to the normal. These defects, occurring among 5,280 experimental animals, did not appear among over 2,000 control animals. The radiation that Bagg gave in these experiments was minimal, being applied for only twelve seconds over the dorsum of the mouse on each of five successive days. The doses would probably not total much more than 100 to 150 r.

As little as 145 r delivered to the ovaries of mice will reduce the fertility of the animals by one-half. The surviving ova either die in

1. Bagg, 1926. Demerec. Flaskamp. Hertwig. Muller, 1933. Perthes.



a short time or are underdeveloped (Martius, 1930). Female mice irradiated with 240 to 280 r become entirely sterile. For the first three to four weeks their matings may prove them fertile. During this period of fertility the size of the litter is reduced and abnormalities of heredity develop in 6 per cent (Snell, 1939; Snell and Ames). Martius (1926) irradiated lightly 5 female mice. Four bore litters, but the litters were small and all the offspring were sterile.

Snell (1933) found that the litters sired by male mice that had been irradiated with 800 r before the onset of sterility were smaller, owing to degeneration of the embryos. He explained this as hypoploidy due to roentgen ray-induced chromosomal aberrations. An interesting avian experiment was carried out by Heim. By giving an erythema dose to the ovaries of hens, he produced cessation of laying for fourteen days. However, when laying was resumed, the chicks born from the eggs were normal. Seven weeks later he gave a full erythema dose, and when laying was resumed, the embryos were either dead or abnormal.

Mutations can be brought about by other means than radiation—for example, by poisoning with certain of the lipid solvents.

The extensive review of Murphy brought together a considerable mass of experimental information published up to that time (1928). Among the total number of reported offspring, underdevelopment occurred in over 30 per cent, early death in over 25 per cent, gross abnormalities in nearly 30 per cent and weakness in 10 per cent. Some of the offspring showed more than one of these abnormalities.

However, not all results are in accordance with Murphy's. Mice irradiated with 12 per cent, 24 per cent or 36 per cent of an erythema dose produced three generations of normal offspring (Krupshi and Eisenberg). Normal litters were obtained from rabbit does after artificial insemination with spermatozoa irradiated at 200 kilovolts (Asdell and Warren). Doses of radiation heavy enough to effect complete sterilization did not produce any hereditary defects among 3,000 descendants (Dobrovolskaia-Zavadskaia).

Drips and Ford, using 5 to 10 per cent of an erythema dose on rats, found no changes produced in the offspring.

Nurnberger concluded that there was no sound experimental evidence for radiation injury of the germ plasm.

There is a considerable amount of data on man, much of it poorly controlled. One of the larger series is that of Jost, who reported on 141 cases in which radiation was used for various pelvic abnormalities. Among the women treated there were 63 pregnancies; 14 of the women aborted; 41 children were born alive, and all but 1 of these were normal mentally and physically. The child excepted suffered from strabismus and myopia, was underdeveloped and did not speak until it was  $3\frac{1}{2}$  years of age.

It has been claimed that temporary sterilization is not injurious to the offspring (Nurnberger; Peller). Pregnancy has been reported in the human being following by three months the intrauterine application of radium (Dawson). Wintz reported that among 500 children of mothers temporarily sterilized none have shown genotypic injuries.

Kaplan reported that of 76 women who had been supposedly sterilized by roentgen irradiation of the ovaries and whose menstruation became reestablished, 44 became pregnant. These produced 47 living children, all of whom were normal. Eight of the 44 aborted. However, it must be remembered that genetic abnormalities, if produced, may not be apparent for several generations.

The reports of Murphy (1928, 1929, 1930) have presented a number of instances of defects produced by radiation injury of the germ plasm. Among 377 radiologists circularized by Hickey and Hall (1927), 37 per cent of the couples were sterile and 4 per cent of 412 children born after employment of a parent in radiation showed abnormalities.

Henshaw, in his article on the biologic significance of the tolerance dose of roentgen rays, and radium, summarizes much of the evidence for genetic injury, concludes the danger is real and feels that the generally accepted tolerance dose of 0.1 r per day may be dangerously large.

#### THE EMBRYO

While there has been some controversy as to the effect of radiation on the germ plasm, there is general agreement as to the deleterious effect of moderate to heavy radiation on the embryo. Although the data in human cases are not entirely in agreement, the preponderance of evidence establishes for the human being the same general conclusions as can be drawn from animal experiments. Not only may death of the embryo result, but various abnormalities may be produced as well. The nature of these abnormalities varies, some being due to the effect on specific sensitive structures, such as the eye (von Hippel and Pagenstecher) or the epiphysial line, others being due to an effect on the blood vessels with failure of adequate nutrition of a given part, as in some of the deformities of extremities. The claim has been made that indirect irradiation may cause late injury to the fetus (Dautwitz), but the evidence for this is completely unsatisfactory. The early references in this field are of little value because of lack of detail, although it was noted that long irradiation would kill the embryos in utero in guinea pigs (Lengfellner, Maurer), that the effects of radiation were more severe shortly before or soon after birth (Krukenberg) and that abortion was produced in dogs by exposure to roentgen rays (Regaud and co-workers). When pregnant rabbits are heavily irradiated at nearly full term, the young either die at birth or within a short time after birth (Lacassagne and Coutard).

Various reports exist as to the effect in man. Some are equivocal and make one wonder whether a chance abnormality of the fetus has been ascribed to radiation. For example there is the case of a woman irradiated during the third and fourth months of pregnancy, whose child died soon after birth and showed intense general edema (von Spindler).

There is no question, however, that abnormalities are produced in the human as well as in the animal embryo in utero by radiation. They fall into two major groups, one of changes in development of the head, the other of abnormalities of the limbs (Murphy and Goldstein). While those of the limbs have been referred primarily to vascular damage, it is possible that some may be caused by damage to the epiphyseal lines. If irradiation of the human pelvis is carried out fairly soon after conception, abortion results. A dose of 600 r at 200 kilovolts is usually sufficient. After the first month (Mayer and co-workers) radiation usually produces fetal injury rather than abortion (Ellinger).

Disturbances of the brain usually occur when radiation is given fairly early in fetal life. Thus, in the rat, the ninth day of pregnancy is the critical period for irradiation of the brain and that for the anlage of the eye is the tenth day (Job and co-workers).

In one series (Murphy) 11 of 24 cases of postconception treatment with radiation terminated in abnormality of the fetus—in the majority of the cases in which this occurred the exposure to radiation took place prior to the fourth month.

One of the largest American series of human embryos irradiated in utero is that of Goldstein and Murphy (1929a). Of 75 children, 38 showed defects or ill health. In 10 the ill health was not due to radiation, but in 28 (37 per cent of the total) it was. Twenty of these 28 showed changes in the central nervous system, and 16 per cent of the group were microcephalic. Goldstein and Murphy (1929b) also reported a case in which microcephalic idiocy followed a cervical treatment of 4400 milligram hours of radium given to the mother for carcinoma during the sixth month of pregnancy.

In 1931 the German national societies interested in heredity and racial hygiene resolved that if a fetus in utero is irradiated the pregnancy should be terminated on eugenic grounds.

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## Notes and News

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**Appointments.**—Paul R. Cannon, of University of Chicago, and Robert A. Moore, of Washington University, St. Louis, have been elected to the American Board of Pathology as trustees at large.

Milton G. Bohrod has been appointed pathologist to the Rochester General Hospital, Rochester, N. Y.

Nicholas W. Popoff has resigned as pathologist to the Highland Hospital, Rochester, N. Y.

**Awards.**—Evarts A. Graham, professor of surgery in Washington University, has received the St. Louis Award given annually by an anonymous donor to the citizen of St. Louis who has made "the most outstanding contribution to the community during the year." The award was made in recognition of Dr. Graham's work on pulmonary and hepatic surgery and his leadership as teacher of students and practitioners.

Howard A. Howe and David Bodian, of the Johns Hopkins University, have been given the Mead Johnson Award by the American Academy of Pediatrics for their work on epidemic poliomyelitis.

The alumni of the New York City Hospital have established the James R. Lisa Award for meritorious research in the laboratories of the hospital.

**Yerkes Laboratories of Primate Biology.**—The name of Yale Laboratories of Primate Biology, Orange Park, Fla., has been changed to the Yerkes Laboratories of Primate Biology, in honor of Robert M. Yerkes, the founder and director emeritus. The laboratories will be conducted by Yale and Harvard universities. The new director is Karl S. Lashley, and Henry W. Nissen continues as assistant director.

**Professorship in Cancer Research.**—The Citizen's Aid Society of Minneapolis has given \$5,500 a year for five years to the University of Minnesota to support what will be known as the George Chase Christian professorship in cancer research. The first incumbent of the position will be John J. Bittner, at present associate director of the Jackson Memorial Laboratory, Bar Harbor, Maine.

**Research in Problems of Sex.**—Applications to the Committee for Research in Problems of Sex of the National Research Council, for financial aid during the fiscal year beginning on July 1, in support of work on fundamental problems of sex and reproduction, should be received before April 1. They may be addressed to the chairman, Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Although hormonal investigations continue to command the interest and support of the committee, preference, in accordance with current policy, will ordinarily be given to proposals for the investigation of neurologic, psychobiologic and behavioral problems of sex and reproduction.

## Book Reviews

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**The Hemorrhagic Diseases and the Physiology of Hemostasis.** Armand J. Quick, Ph.D., M.D., associate professor of pharmacology, Marquette University School of Medicine, Milwaukee. Volume 17. Pp. 340, with 23 figures. Price \$5. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

This monograph is an expansion of the author's Beaumont Lectures, delivered in 1941, in which he brought together "the contributions of the laboratory, especially in the field of the coagulation of the blood, and the clinical information on the principal hemorrhagic diseases." The first seven chapters are devoted to the agents concerned in coagulation of the blood and hemostasis: thrombin, prothrombin, fibrinogen, thromboplastin, platelets and anticoagulants. The following hemorrhagic diseases and conditions are described fully in the light of recent developments: thrombopenic purpura; hereditary hemorrhagic diathesis: pseudo-hemophilia and telangiectasia; afibrinogenemia; hemophilia; hemorrhagic diathesis of avitaminosis K; obstructive jaundice, biliary fistula and damage of the liver. The history, the nature and the actions of heparin and vitamin K are discussed fully. A chapter in the story of vitamin K is summarized as follows: "It will be difficult to find in the annals of medical history a story comparable to the quest of vitamin K. After several groups vied with each other to win the mythical laurels of being the first to synthesize vitamin K, they found at the end of the race, a bottle gathering dust on the laboratory shelf containing a simple chemical, methyl naphthoquinone, which was soon to claim the lion's share of attention. But odd things do occur and no one could predict that a synthetic chemical should show a vitamin activity even greater than the natural product on which mankind and all the higher animals have depended through the ages for the normal coagulation of the blood." There are succinct statements on the classification of hemorrhagic diseases and on hemostasis. In the appendix are described tests, selected on the basis of clinical value, for the study of these diseases. There are good lists of references at the ends of the chapters. The illustrations, mostly charts and curves, are instructive. The author, known for his researches on fundamental problems of blood coagulation, summarizes competently and clearly the present state of knowledge of the hemorrhagic diseases from all points of view. The book will be a landmark in its field.

## Books Received

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ANNUAL REPORT FOR THE YEAR 1941 OF THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG. E. H. Cluver, M.A., M.D., B.Ch., D.P.H., F.R.S.I., director.

MEDICAL PARASITOLOGY. James T. Culbertson, assistant professor of bacteriology, College of Physicians and Surgeons, Columbia University. Pp. xvi + 285, with 21 plates. Price \$4.25. New York: Columbia University Press, 1942.

NEW YORK STATE DEPARTMENT OF HEALTH, EDWARD S. GODFREY JR., M.D., COMMISSIONER. Annual Report of the Division of Laboratories and Research, Augustus B. Wadsworth, M.D., director. Pp. 117. Albany, 1941.

THE SIMS-WOODHEAD MEMORIAL LABORATORY RESEARCH BULLETIN FOR 1939 AND 1940. Papworth, England: Pendragon Press.

CHANGES IN THE KNEE JOINT AT VARIOUS AGES WITH PARTI-REFERENCE TO THE NATURE AND DEVELOPMENT OF DEGENERATIVE JOINT DISEASE. Granville A. Bennett, M.D., associate professor of pathology, Harvard Medical School. Hans Waite, M.D., research fellow in medicine, Harvard Medical School, and graduate assistant in medicine, Massachusetts General Hospital. Walter Bauer, M.D., associate professor in medicine, Harvard Medical School, physician to the Massachusetts General Hospital, and director of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases. Pp. 97, with 31 plates. Price \$2.50. New York: The Commonwealth Fund, 1942.

SURGICAL PATHOLOGY. William Boyd, M.D., LL.D., M.R.C.P.Ed., F.R.C.P. Lond., Dipl. Psych., F.R.S.C., professor of pathology, University of Toronto. Fifth edition, thoroughly revised. Pp. 843, with 502 illustrations, 16 colored plates. Price \$10. Philadelphia: W. B. Saunders Company, 1942.

CONSTITUTION AND DISEASE, APPLIED CONSTITUTIONAL PATHOLOGY. Julius Bauer, M.D., professor of clinical medicine, College of Medical Evangelists, Los Angeles, Calif., and formerly professor of medicine of the University of Vienna. Pp. 208. Price \$3.50. New York: Grune & Stratton, 1942.

THE INTERNATIONAL CANCER RESEARCH FOUNDATION. Report of Activities During 1941. Pp. 157. Lincoln-Liberty Building, Philadelphia.

## METASTASIS OF MIXED TUMORS OF THE SALIVARY GLANDS

R. M. MULLIGAN, M.D.

DENVER

From a survey of the literature on mixed tumors of the salivary glands which were proved at autopsy to have metastases it would seem at first glance that the development of secondary growths from them is uncommon as contrasted with the rather high recurrence rate. Although this paper is concerned only with cases authenticated at reasonably complete autopsies, several factors suggest that metastasis of mixed tumors of the salivary glands is more frequent than currently assumed. These factors are: (1) reports of cases with strong presumptive clinical evidence of metastases; (2) reports of cases in which biopsy of lesions distant from the primary site during life showed growths that resembled the primary tumor in every histologic detail; (3) occasional partial autopsy reports of such instances; (4) the difficulty of following the patient to the time of death because of the long clinical course of the disease; (5) the possibility of death of the patient before dissemination could occur; (6) the ever present obstacle of obtaining permission for an autopsy from the relatives of a patient who has suffered from the operative and irradiation aftermaths of treatment for the primary tumor and its recurrences, and (7) the possible failure to record cases with metastases observed at complete autopsies.

In the present analysis of recorded instances of metastasizing mixed tumor of the salivary glands, the following details will be considered: sex, age, location of the primary tumor, duration of the primary tumor before it was first seen by the author, its largest size, duration of life after it was first seen, number of recurrences, location of metastases, histologic characteristics of the primary tumor and of the metastases and the immediate cause of death. When any of these details is not given, it was not found in the original reports.

Although the original reports reveal a rather wide variety of names and thus an apparent dissimilarity of the tumors which are now being considered under the heading of metastasis of mixed tumors of salivary glands, it is thought from the information available that the cases studied are bona fide instances of such metastasis. The histologic variation in

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a group of mixed tumors of the salivary glands, the different descriptive terms which may be employed if their fundamental nature is forgotten and the confusion in the minds of persons who reported cases in the older literature, especially when the eminent authorities of the day disagreed so diametrically on the origin and nomenclature of these tumors, have been borne in mind in the analysis.

#### REVIEW OF CASES RECORDED IN THE LITERATURE

Förster<sup>1</sup> reported the case of a woman 34 years old who had had a tumor of the right parotid region for eight years. It had attained the size of a goose egg. There was no recurrence, as the patient died from pneumonia a few days after her first visit. Metastases were found in the petrous portion of the right temporal bone, in the lungs and in the pleurae. Sarcomatous, cartilaginous and mucoid tissue comprised the tumor.

Tommasi<sup>2</sup> described a woman 51 years old who had had a tumor in the right submaxillary region for eight years; it had reached the size of a hen's egg. No recurrence was noted. Metastases were discovered in the cervical lymph nodes, pleurae, lungs, liver (12 pounds [5.4 Kg.]), left kidney, pelvic peritoneum, ovaries and dura. They were composed of mixed tumor with a cylindromatous structure.

Chiari<sup>3</sup> observed a man of 45 years with a tumor of the left parotid region of two years' duration, which became the size of a man's fist. The man died of erysipelas and pneumonia three weeks later, without recurrence. The only metastases were in the lungs. The tumor consisted of glandlike tissue and fibrous connective tissue, similar to the so-called cylindroma of the parotid region and orbit.

Griffini and Trombetta<sup>4</sup> saw a woman of 56 years with a tumor of the submaxillary gland which had been present for sixteen years. Metastases were found in the cervical and bronchial lymph nodes, the lungs and the pleurae. The tissues revealed a cellular mixed tumor with a rich content of cartilage and an alveolar arrangement of tumor cells.

Zahn<sup>5</sup> discussed a man 49 years old who had had a tumor of the left submaxillary region for two months. He died of lobar pneumonia four months later, without recurrence. Metastases involved the manubrium of the sternum, the right seventh rib, the second lumbar vertebra and the right hip joint, femur and pubis. The tumor extended into the right anterior cranial fossa, the mouth, the organs of the neck and the right orbit. The tumor cells were in an alveolar pattern and were associated with heavy fibrous tissue. The cells centrally placed were degenerated, and those peripherally located were well preserved.

LeDentu<sup>6</sup> examined a woman 61 years old in whom there had been a tumor of the left parotid region for thirty years, which had attained the bulk of 1,300 Gm., or more than half the size of the patient's head. At autopsy, twenty-one days later,

1. Förster: *Wien. med. Wchnschr.* **8**:481, 1858.

2. Tommasi, C.: *Virchows Arch. f. path. Anat.* **31**:111, 1864.

3. Chiari, H.: *Wien. med. Presse* **21**:746, 1880; *Med. Jahrb.* **11**:1, 1881.

4. Griffini, L., and Trombetta, F.: *Arch. per le sc. med.* **7**:71, 1883-1884; cited by Kornblith.<sup>13</sup>

5. Zahn, F. W.: *Virchows Arch. f. path. Anat.* **117**:1, 1889.

6. LeDentu, A.: *Études de clinique chirurgicale*, Paris, Masson & Cie, 1892, p. 154; cited by Heinike, H.: *Ergebn. d. Chir. u. Orthop.* **6**:239, 1913.

metastases were found in the lungs, liver and the meninges. The tissue consisted of fibrous septums about irregular alveoli. Some areas were in the process of liquefaction.

Barozzi and Lesne<sup>7</sup> saw a woman 34 years old who had had a tumor of the left submaxillary region for twelve years. Metastases were noted in the cervical and the tracheal lymph nodes and in the lungs. The tissue showed connective tissue surrounding islands of myxoepithelioma and the pattern of cylindroma.

LeClerc<sup>8</sup> described a man 65 years old who had had a tumor of the right parotid gland for four years, which became the size of an orange. Death was caused by extension of the tumor to the right zygoma, the right middle cerebral fossa, the right temporal lobe and the right orbit. Metastases were found in the lungs and pleurae. He called the tumor a malignant glandular epithelioma.

Rispa and Samiac<sup>9</sup> observed a 62 year old woman with a tumor in the left parotid region. She died from the effects of its extension to the dura six weeks later. Metastases were present in the cranial bones. The microscopic diagnosis was cylindroma.

Partsch<sup>10</sup> saw a woman 31 years old who had had a tumor in the right parotid region for sixteen years; it had reached the size of a hen's egg. She lived for thirteen years, and two recurrences developed. Metastases were noted only in the pleurae after death from cellulitis of the right cheek and neck, which developed after an operation for the second recurrence. The specimen was a well encapsulated mixed tumor of cylindromatous structure with extensive hyaline and mucous transformation.

Garvey<sup>11</sup> reported the case of a woman 31 years old who had had a tumor in the left parotid region for eight years, which attained the size of an olive. She died eighteen months later of dilatation of the right side of the heart with passive congestion of the viscera, after experiencing two recurrences in the course of the disease. Metastases were found in the vertebrae, femurs, skull, sternum, clavicle, lungs, pleura, liver, retroperitoneal lymph nodes, adrenals and spinal meninges. The tumor also extended into the left gasserian ganglion. It was diagnosed as adenocarcinoma.

Brunschwig<sup>12</sup> discussed a woman 60 years old who had had a tumor of the right sublingual gland for nineteen years. She had one recurrence. A few days after her last admission to the hospital she died from the effects of the tumor. The lungs and pleura were involved by metastases. The tumor extended to the mandible, the mouth and the larynx. The four photomicrographs indicate its mixed tumor nature. The author commented: "In all these sections made from tissue removed at intervals over a period of 19 years, the character of the epithelial elements remains unchanged."

Kornblith's first case<sup>13</sup> was that of a man 55 years old with a tumor in the right submaxillary region the size of a plum. Three recurrences marked the total

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7. Barozzi and Lesne: *Bull. Soc. anat. de Paris* **72**:266, 1897; cited by Kornblith.<sup>13</sup>

8. LeClerc, G.: *Lyon méd.* **101**:864 and 900, 1903.

9. Rispa and Samiac: *Toulouse méd.* **8**:121, 1906.

10. Partsch, F.: *Deutsche Ztschr. f. Chir.* **183**:269, 1923.

11. Garvey, J. L., in Stone, W. J.: *Contributions to Medical Science, Dedicated to Aldred Scott Warthin*, Ann Arbor, Mich., George Wahr, 1927, p. 661.

12. Brunschwig, A.: *Surg., Gynec. & Obst.* **50**:407, 1930.

13. Kornblith, B. A.: *Virchows Arch. f. path. Anat.* **286**:74, 1932.

course. Slightly over four years after his first visit he died. The lungs and the liver contained metastases. The structure of a cylindroma was evident. Small, delicate epithelial cells were arranged in nests in a connective tissue or mucous tissue stroma. Hyaline masses were in the centers of the cell groups.

Fitzwilliams<sup>14</sup> observed a woman 48 years old in whom a tumor of the left submaxillary region had been present for five years. She had two recurrences. Autopsy, four months after the author first saw her, disclosed metastases in the cervical lymph nodes on the left side and in the pleurae and extension to the mandible. The structure was that of a cellular salivary gland tumor. Polygonal cells formed small solid masses; columnar cells formed tubes and alveoli; cells were drawn out in a mucinous stroma; a small number of mitotic figures was seen.

Boemke<sup>15</sup> examined a woman 35 years old in whom a tumor of the hard palate had been present for five years. Two days after operation for removal of the first recurrence, she died of bronchopneumonia. The tumor and the metastases in the lungs, the liver and the right kidney was a cylindroma with the formation of hyaline cylindromatous structures.

Olson<sup>16</sup> outlined the case of a woman 49 years old who had a tumor of the right side of the hard palate and metastases in the bronchial and mediastinal lymph nodes, the lung, the pleurae, the spleen and the liver, which he designated a mixed tumor.

Kornblith's second case<sup>17</sup> was that of a woman 33 years old who had had a tumor in the right parotid region for fourteen years. It had attained the size of a plum. She had one recurrence and died a few months after the author first saw her of the effects of the tumor, which extended to the mandible, the zygoma, the temporal bone, the middle and posterior cranial fossae and the rhinopharynx; compressed the cerebrum, the pons and the medulla; and metastasized to the cervical lymph nodes, the lungs, the pleura of the left diaphragm and the liver. The tumor was composed of "branching, interlacing, homogeneous, hyaline, mucoid, cylindrical rows of globules lined by small cells in single or double rows and resembling basal cells." It had the structure of a cylindroma.

McKnight<sup>18</sup> observed a Negro boy 15 months old with a tumor of the left parotid region present since birth; it had reached the size of a plum. One recurrence followed operative removal, ten months after which the boy died from the effects of the tumor. Metastases were discovered in the skull bones, the left temporal lobe of the brain and the lungs. The tumor was diagnosed as adenocarcinoma.

Livingston<sup>19</sup> reported the case of a Negro man 44 years old who had had a tumor in the right parotid gland removed nine years prior to his last admission. Six years after that operation a recurrence was seen. Ten weeks after his last admission he died of lobar pneumonia. The lungs, liver, spleen, left adrenal, gallbladder, sternum and left second rib were involved by metastases. The tumor was diagnosed as "teratoma (mixed tumor)." It was composed of fairly uniform, lightly staining polyhedral cells. Mitotic figures were present but not numerous. In places the cellular structure presented a mucoid appearance.

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14. Fitzwilliams, D. C. L.: *Lancet* 2:769, 1935.

15. Boemke, F.: *Centralbl. f. allg. Path. u. path.-Anat.* 64:129, 1936.

16. Olson, G. W.: *Laryngoscope* 47:252, 1937.

17. Kornblith, B. A.: *J. Mt. Sinai Hosp.* 6:38, 1939.

18. McKnight, H. A.: *Am. J. Surg.* 45:128, 1939.

19. Livingston, S. K.: *Am. J. Roentgenol.* 44:887, 1940.

Perrin<sup>20</sup> described a man 35 years old who had had a tumor of the right parotid region for seven years and who died four months later. Metastases were found in the lungs, liver, pleurae, lumbar vertebrae, subcutaneous tissue, omentum and retroperitoneal tissue. A matrix of hyalin and mucocartilage contained cell strands and aggregates of glands of juvenile cubical epithelium. In some areas were hollow branching formations of neoplastic epithelium with hyperchromatic nuclei.

A number of case reports of histologically proved distant metastases, such as those by Budde<sup>21</sup> and by Gueit and Puech,<sup>22</sup> have appeared in the literature. Several investigators have described cases with histologically substantiated metastases in lymph nodes. Others have observed cases in which all other primary sites were eliminated on clinical grounds. In the tumor clinic here 2 patients have been followed who had metastases in the lungs as shown by roentgen examination, with probable origin in the salivary glands, but unfortunately permission for autopsy was not obtained in either case. None of these cases is included in the present report, since the studies made of them are incomplete in that other possible primary sites of origin were not eliminated.

#### REPORT OF A CASE

A bookkeeper 54 years old was admitted to the Colorado General Hospital on March 22, 1942. For twelve years he had had a tumor the size of a pea anterior to the left ear in the region of the parotid gland. In the course of a year this enlarged to the size of a lemon. Six months before admission, while on a fishing trip he fell down in a boat. Following this, he experienced lumbar pain which radiated down the right leg. He received a "spinal adjustment" and massage of the parotid tumor. Temporary lessening of the pain and some decrease in the size of the tumor were noted. For four months he had paralysis, first of the right and then of both legs, necessitating confinement to bed. He also had uncontrollable dribbling of urine. For three months he tolerated only a soft diet because of gagging caused by ingestion of solid food. For four days he was drowsy.

On examination the temperature was 98 F.; the pulse rate, 110; the respiratory rate, 20. The blood pressure was 116 systolic and 80 diastolic. In addition to drowsiness and gagging at the sight of a tongue blade (making pharyngeal examination impossible), the significant findings were: anterior to the left ear, a non-tender hard lemon-sized mass attached to the underlying tissue but not to the skin; false dentures; bilateral foot drop with paralysis of the flexor muscles and marked paresis of the extensor muscles; marked incoordination in the heel to knee test; marked weakness in the thigh and hip muscles; absence of patellar and achilles reflexes; bilateral absence of the Babinski sign; normal sensation and vibratory sense. On neurologic consultation the diagnosis was: compression of the cauda equina by a metastatic tumor.

The hemoglobin was 15.0 Gm. (Newcomer); the erythrocyte count, 5,280,000; the total leukocyte count, 12,160, with polymorphonuclear neutrophils 68 per cent, lymphocytes 30 per cent and monocytes 2 per cent. The sedimentation rate was 22 per cent in one hour. The urinalysis, the Wassermann test of the blood and

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20. Perrin, T. L.: *Arch. Path.* **33**:930, 1942.

21. Budde, M.: *Zentralbl. f. Chir.* **49**:1888, 1922.

22. Gueit and Puech: *Bull. Soc. d. sc. méd. et biol. de Montpellier* **4**:52, 1922-1923.

the Eagle test gave negative results. The spinal fluid contained no cells; the sugar content was 72 mg. and the protein content 88 mg. per hundred cubic centimeters; the Wassermann reaction was negative, as were the gold curve and the culture. The sugar content of the blood was 99 mg. and the nonprotein nitrogen content 39 mg. per hundred cubic centimeters.

Roentgen examination of the skull revealed no definite change; that of the chest, accentuated hilar and bronchial markings, round areas of increased density in the middle thirds of the lung fields and accentuation of the aortic arch; that of the pelvis and of the lumbar part of the spinal column, extensive destruction with wedging and anterior displacement of the fourth lumbar vertebra, erosion of the body of the third lumbar vertebra and ballooning of the intervertebral disks in the lumbar part of the spinal column, general atrophy of all bones visualized, mottling of the femur and of the pelvic bones; that of the gastrointestinal tract, no definite evidence of an organic lesion in the stomach or the duodenum. There was a marked six hour gastroduodenal residue.

He continued to be drowsy, became irrational and had hallucinations and delusions. The temperature ranged between 98 and 100 F.; the pulse rate, between 90 and 120. He was still unable to tolerate any but liquid food and was nauseated. He was seen by the members of the staff of the Bonfils Foundation Tumor Conference March 25. The impression was one of widespread metastases with origin possibly in the left parotid region. A punch biopsy, suggested then, was made April 2. Histologic examination revealed groups of polyhedral cells with scanty acidophilic cytoplasm and dark oval or round nuclei. Many mitotic figures were seen. The cells were occasionally grouped to form tubules containing pale basophilic material. April 7 the temperature rose to 103 F., the pulse rate to 170 and the respiratory rate to 34. Moist rales were heard throughout the chest. The patient died April 8, seventeen days after admission and six days after biopsy.

*Postmortem Examination.*—Autopsy, sixteen and one-half hours after death, disclosed a bulging tumor, 6 by 4.5 cm., in the left parotid region. On section this was hard, glistening, yellow-white and studded with pinpoint gelatinous areas. At its lower pole was a red-yellow firm 8 mm. nodule.

Infiltrating beneath the pleura of the anterior ends of the fifth and sixth ribs on the right and the third and seventh ribs on the left was a pink-gray nodular tumor. The largest area was 2.5 cm. The overlying pleura was smooth and intact, although the shafts of the corresponding ribs were invaded.

The heart weighed 380 Gm. The foramen ovale was anatomically patent. The septum primum covered the posterior half and overlapped the septum secundum, but a slit 1 cm. long was demonstrated by probing. The septum primum was marked by six fenestrations, each 0.5 mm. in diameter, on its anterior superior edge. The valves all had thin, delicate leaflets. The thickness of the right ventricle was 3 to 4 mm.; that of the left ventricle, 15 to 18 mm. The coronary arteries in the first two thirds of their extent were stiffened by yellow and white intimal plaques, but no occlusion was seen.

The right lung weighed 810 Gm.; the left, 610 Gm. The surfaces were dark red and smooth and finely mottled with black. Multiple tumor nodules bulged beneath the pleura. A cut section of the posterior four fifths of the lungs was dark red, finely mottled with black and noncrepitant; the anterior one fifth was cushiony, pink-gray, subcrepitant. The anterior edges and apexes showed emphysematous bullae, 4 to 5 cm. in diameter. The tumor nodules, most of them subpleural, 7 to 25 mm. in diameter, were discrete, rounded, firm, red-yellow and

studded with pinpoint glistening gray dots. The trachea and bronchi contained thick gray and yellow and frothy pink-gray fluid.

The stomach was distended with air and about 1,000 cc. of glairy gray-yellow opaque fluid. The mucosa was moderately flattened, pale and gray-yellow. Beneath the mucosa of the colon were gray, red-rimmed nodules up to 5 mm. in diameter. In the sigmoid colon was a pedunculated 1 cm. polyp with a thin stalk.

The liver weighed 1,520 Gm. The cut surface was firm and brown-red. It contained seven pink-white to red-yellow nodules, 5 to 20 mm. in diameter.

As to the skeleton, the anterior end of the bony portion of the left seventh rib was fractured through expansion of its medulla by tumor invasion. The first, second, third and fourth lumbar vertebrae were removed in right hemisection. The body of the second was softened and mushy. The fourth was almost completely destroyed by friable glistening red-yellow tumor, which extended symmetrically on either side beneath the iliopsoas muscles and encroached on the cauda equina.

The brain weighed 1,800 Gm. The convolutions were flattened. Routine transverse sections through it revealed no gross abnormalities.

*Microscopic Examination.*—The significant findings aside from those in the tumor and its metastases were: early bronchopneumonia, chronic passive congestion of the lungs and the liver, early acute esophagitis, melanosis and noncancerous polyp of the colon, moderate adipose tissue replacement of the pancreas and acute lymphadenitis of the mediastinal nodes.

*Tumor of the Left Parotid Gland (A and B in figure):* The tumor cells were polyhedral. Their cytoplasm was acidophilic. The nuclei were round or oval and had a heavy stippled chromatin content. Mitotic figures were evident. Large and small cylinders of amorphous basophilic material were scattered among the tumor cells, which were arranged in plaques of varying size set in a pale basophilic stroma enclosing a few flat spindle cells. The basophilic stroma was separated into islands by broad strands of collagenous connective tissue, in which a rare focus of pigmented macrophages was seen. The tumor impinged on the hilus of a well preserved lymph node, adjacent to which was tissue of the normal left parotid gland (A in figure). In the substance of the lymph node were several small epithelial tubules (C in figure) which were similar in their structure to the ducts of the nearby parotid gland. The inclusion of tubules in this fashion was described by Neisse.<sup>23</sup>

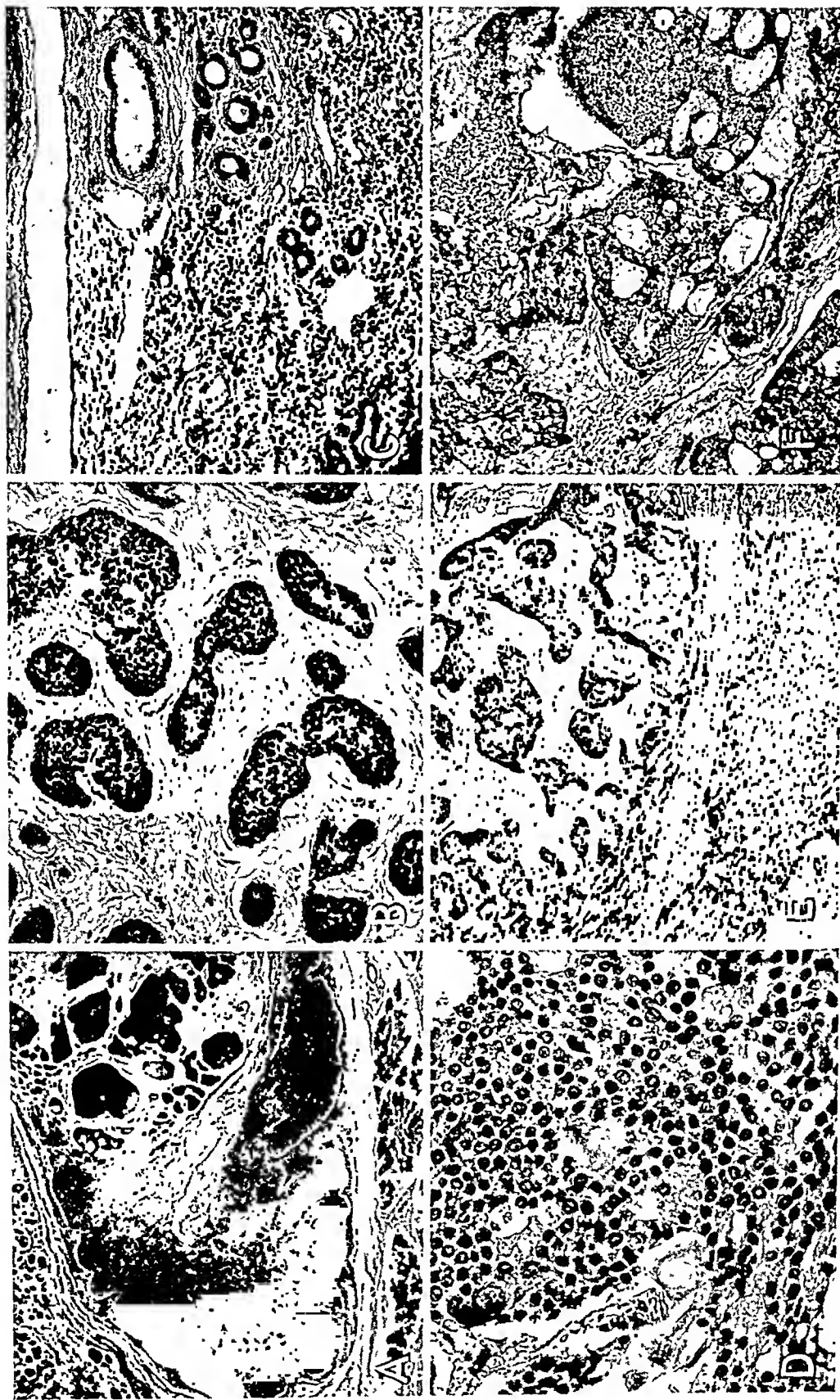
*Metastases in the Lung, Liver and Kidney (D and E in figure):* These consisted of polyhedral cells with acidophilic cytoplasm and round or oval nuclei with dense stippled chromatin content, among which were scattered mitotic figures. The tumor cells were supported on a delicate, well vascularized stroma. Many large and small cylinders of basophilic material were surrounded by tumor cells. The nearby lung and liver parenchyma was compressed and atrophied.

In the kidney was a focus of tumor with a structure like that of the growths in the lungs and the liver. A mass of tumor cells plugged a neighboring vein.

*Fourth Lumbar Vertebra (F in figure) and Adjacent Soft Tissue:* The tumor cells were identical with and arranged in patterns like those in the other metastases except that wide areas of mucoid degeneration were evident within the cylinders among them and in the zone surrounding islands of tumor cells. Adjacent to this outer zone of mucoid change, heavy bands of interlacing connective and collagenous tissue contained blood vessels, nerve trunks, many pigmented macrophages and two small islands of bone.

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23. Neisse, R.: Anat. Hefte 10:287, 1898.



*A*, impingement of the primary tumor on the hilus of a lymph node. Note the normal parotid gland just beneath the lower edge of the node.  $\times 12$ . *B*, detail of primary tumor.  $\times 125$ . *C*, epithelial tubules like those in the parotid gland caught in the hilar edge of the lymph node seen in *A*.  $\times 125$ . *D*, detail of a tumor metastasis in the lung.  $\times 250$ . *E*, tumor nodule with compression of the liver parenchyma in the lower half of the field. Note the periportal area in the lower left hand corner.  $\times 50$ . *F*, metastasis in the fourth lumbar vertebra.  $\times 50$ .

The final anatomic diagnosis was: cancerous mixed tumor of the left parotid gland with metastases to the pleurae, lungs, liver, kidneys, ribs and lumbar vertebrae; coronary arteriosclerosis; cardiac dilatation; patent foramen ovale; cerebral edema; chronic passive congestion of the lungs and liver; bronchopneumonia; acute lymphadenitis of the mediastinal lymph nodes; acute esophagitis; noncancerous polyp and melanosis of the colon.

#### ANALYSIS OF DATA COLLECTED IN TWENTY-ONE CASES

In 20 cases the age at which the patient was first seen by the author varied from 31 to 65 years. Eight males and 13 females were afflicted. In 12 cases the tumor was in the parotid region; in 6, in the submaxillary region; in 2, in the hard palate, and in 1, in the sublingual gland. The duration of the tumor before it was first seen by the author in 19 of the cases was two months to nineteen years. In 11 cases the largest size ranged from that of an olive to more than half the size of the head. The duration of life after the patient was first seen by the author in 17 cases was from two days to thirteen years. The recurrences ranged from none to three. Of the entire series, metastases were present in the lungs in 18, in the pleurae in 12, in the liver in 10, in the bones in 8, in lymph nodes in 6, in the kidneys in 3 and in the spleen in 2. In 8 cases the term "cylindroma" was employed in the histologic description of the tumor. In 4 cases this same appearance was present if one may judge from the description given. In 5 "mixed tumor" was the designation of the neoplasm. In 2 "adenocarcinoma" was the term used. In the 2 remaining cases "malignant glandular epithelioma" and "alveolar sarcoma" were the names given to the growths.

#### COMMENT

The facts presented in the foregoing analysis of the data in 21 cases suggest that the following points should be remembered when a patient in the fourth, fifth or sixth decades of life has a tumor of a salivary gland: duration of the growth; size of the tumor at various stages in its evolution; a consideration of the metastases as they may affect the lungs, pleurae, liver, bones, lymph nodes, kidneys, spleen and other sites; a careful determination of the extension of the tumor, particularly into the meninges and the brain; some thought of the possible relationship of a cylindromatous type of structure in the primary tumor to the development of metastases, and a thorough examination of the patient to rule out metastases if an operation on or irradiation of the primary tumor is contemplated.

#### SUMMARY

A review has been made of 20 case reports of mixed tumors of the salivary glands in which metastases were found at autopsy.



# COMMERCIAL LEAD AS A POSSIBLE INCITING FACTOR IN BRONCHIOGENIC CARCINOMA

## REPORT OF TWO CASES

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It is evident from the literature that little or no emphasis is placed on commercial lead as a factor in the production of primary carcinoma of the lung. Probably not enough care is directed to the incidence of carcinoma in occupations that deal with commercial lead. Lanza<sup>1</sup> emphasized the inadequacy of information on the mortality from lead poisoning and the absence of information relative to the morbidity. He stated that there is no doubt that in many cases lead poisoning is reported on the death certificate as some organic disease, no mention being made of the underlying plumbism. Lanza<sup>1</sup> also explained why the manifestations of plumbism are less frequently encountered in the major lead industries, such as smelters and battery and paint factories, but are seen in industries where the contact with commercial lead is just one step in the manufacture. This is because the heads of the major industries are fully cognizant of the hazards of lead taken into the body and utilize precautionary measures against it, while those of industries and occupations in which the contact with lead is only a step in the process tend to underestimate the hazard or to ignore it completely. It is likewise difficult to obtain accurate information in regard to such varied occupations as those of painters, printers and plumbers.

The widespread and increasing use of lead throughout industry makes it of more and more concern to the physician and the public health official. Some of the industries in which lead is a hazard are: lead mining; lead smelting; lead refining; lead fabrication; hot lead processes; lead burning; lead soldering; lead tempering; plumbing; iron and other founding; buffing and polishing; storage battery, paint, glass, rubber and chemical industries; the applying and removing of lead paints, enamels and glazes, and typographic trades, such as type founding, electrotyping and stereotyping. According to Lanza,<sup>1</sup> most of the industrial exposure arises from the inhaling of dust and fumes into the lungs.

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1. Lanza, A. J.: J. A. M. A. **104**:85, 1935.

Several writers have reported pulmonary carcinoma in workers of the glass industry, in which lead is used. Lead is employed mainly in the manufacture of flint glass, which is used in table and other fine glassware and in high grade optical lenses. Lead oxide is commonly used in flint glass in the proportion of 33 per cent. Boyd,<sup>2</sup> as early as 1887, reported cancer of the lung in a glass blower. Gutzeit<sup>3</sup> reported bronchiogenic carcinoma in a glass maker. Klotz<sup>4</sup> reported carcinoma of the lung in 24 persons, among whom were 2 glass workers, 1 miner and 1 polisher. Metal polishes often contain lead. Seyfarth<sup>5</sup> noted that carcinoma of the lung occurred with special frequency among cigar makers, metal workers, type setters and printers. According to Kohn,<sup>6</sup> some of the intoxications associated with tobacco have been found to be due to lead.

Rosedale and McKay<sup>7</sup> reported a study of the occupational incidence of carcinoma of the lung. Forty-three patients, or 75.4 per cent of the 57 persons studied, were employed in occupations exposing them to dust or other irritating atmospheric factors, and 9 of the 43 had been working more or less with lead in one form or another. Lanza<sup>1</sup> pointed out that lead poisoning is not uncommon among industrial workers who are not engaged in handling lead but who through negligence are exposed to dust or fumes from lead processes not properly protected.

There is much difference of opinion as to the role played by the various dusts and fumes in inciting carcinoma of the lung. The view that tuberculosis and influenza are factors in the production of bronchiogenic carcinoma is gradually being discarded. Weller<sup>8</sup> summarized the causes by stating that a variety of chronic factors are potent in the production of carcinoma of the lung in various degrees in different persons, depending on the degree of intrinsic predisposition.

It is generally agreed that inhaling radioactive dust is an important etiologic factor in carcinoma of the lung. Saupe,<sup>9</sup> in a critical review, concluded that the high incidence of pulmonary carcinoma in the Schneeberg cobalt miners in Europe over a long period of years was due to inhalation of radioactive mine dusts. Saupe<sup>9</sup> also emphasized that carcinoma develops after prolonged exposure to radioactive dust, ranging from ten to forty-five years, the average age of incidence being 55 years.

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2. Boyd, M. A.: *Lancet* **2**:60, 1887.

3. Gutzeit, K.: *Ztschr. f. Krebsforsch.* **19**:30, 1922.

4. Klotz, O.: *Canad. M. A. J.* **17**:989, 1927.

5. Seyfarth, C.: *Deutsche med. Wchnschr.* **50**:1497, 1924.

6. Kohn, H.: *Deutsche med. Wchnschr.* **52**:447, 1926.

7. Rosedale, R. S., and McKay, D. R.: *Am. J. Cancer* **26**:493, 1936.

8. Weller, C. V.: *Arch. Path.* **7**:478, 1929.

9. Saupe, E.: *Zentralbl. f. inn. Med.* **54**:825, 1933.

Weller,<sup>8</sup> Fried,<sup>10</sup> Vinson,<sup>11</sup> Rice,<sup>12</sup> Stein and Joslin,<sup>13</sup> Matz,<sup>14</sup> Rosahn,<sup>15</sup> Ochsner and DeBaKey,<sup>16</sup> Menne and Anderson<sup>17</sup> and Karsner<sup>18</sup> could find no definite cause for bronchiogenic carcinoma except for that in the Schneeberg cobalt miners, which is thought to be due to the radioactive dust in the atmosphere of the mines.

In chemical character lead and radium are closely allied, since they belong to the same chemical family. Their atomic numbers are in the same range in the periodic table, radium being 88, radon, 86, radium D 82 and lead 82. This further denotes similar chemical properties. In fact, radium is only one member of the family of linear descendants from the parent element, uranium, to the various radioactive isotopes of lead, such as radium B, thorium B, actinium B and radium D, or radiolead (Hoffman and Strauss). According to Rutherford and co-workers,<sup>19</sup> radium D is so closely allied physically and chemically to lead that it cannot be isolated in the analysis of a uranium mineral. They emphasized that if any radium is present in the ore from which lead is prepared, radium D will be separated with the lead and remain with it throughout the process of purification. They stated that the life span of radium D is about twenty-five years, meaning that the radioactivity of commercial lead decreases with age. Smith and associates<sup>20</sup> recently reemphasized that commercial lead is by far the most radioactive of all the common metals. Certain lots of industrial lead contain more radioactive substances than others, depending somewhat on where the ore is mined.

The toxic manifestations of lead and radium are strikingly similar; radium, being more toxic, produces a more marked effect. Smith and co-workers<sup>20</sup> and others have shown that colloidal lead preparations containing radioactive lead when injected into laboratory animals caused ulceration of the jaw, degenerative changes in the liver and the kidneys and inflammatory infiltrations of the lungs. The lesions of the bones, such as rarefaction due to necrosis and osteitis, were similar to those

10. Fried, B. M.: *Medicine* **10**:373, 1931.

11. Vinson, P. P.: *J. A. M. A.* **107**:258, 1936.

12. Rice, C. M.: *J. Lab. & Clin. Med.* **21**:906, 1936.

13. Stein, J. J., and Joslin, H. L.: *Surg., Gynce. & Obst.* **66**:902, 1938.

14. Matz, P. B.: *J. A. M. A.* **111**:2086, 1938.

15. Rosahn, P. D.: *Arch. Path.* **29**:649, 1940.

16. Ochsner, A., and DeBaKey, M.: *Arch. Surg.* **42**:209, 1941.

17. Menne, F. R., and Anderson, M. W.: *J. A. M. A.* **117**:2215, 1941.

18. Karsner, H. T.: *Human Pathology*, ed. 6, Philadelphia, J. B. Lippincott Company, 1942, p. 479.

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20. Smith, F. L.; Rathmell, T. K., and Marcil, G. E., in Piersol, G. M., and Bortz, E. L.: *The Cyclopedia of Medicine, Surgery and Specialties*, ed. 2. Philadelphia, F. A. Davis Company, 1940, vol. 8, p. 811.

produced by radium. Crawford and associates,<sup>21</sup> experimenting with cats, found that those which received colloidal lead alone generally remained in good clinical condition without loss of weight and without anemia. However, the cats which received both colloidal lead and radiation had more pronounced manifestations, such as loss of weight, anemia and infections, than the group of cats that received lead alone or radiation alone. The tissues examined from the cats which received only lead showed no characteristic changes. These observations are in accord with those of Aub<sup>22</sup> and the opinion of most pathologists that there is no specific lesion produced by lead with any degree of frequency.

Even small amounts of radioactive substances are hazardous, particularly when inhaled. Rajewski<sup>23</sup> emphasized that only one-fifth as much radium is required to produce the same effects when inhaled or injected intravenously as when taken by mouth. Likewise, Aub<sup>22</sup> stated that lead inhaled is more toxic than that taken by mouth. Stevens,<sup>24</sup> in reporting a case of radium poisoning following intravenous injection of radium chloride for Hodgkin's disease, pointed out that the percentage of permanent fixation of radium in the tissues is vastly greater when the chemical is injected directly into the blood stream than when it is taken orally. From a clinical standpoint, lead and radium poisoning are also similar. Lead<sup>22</sup> and radium<sup>24</sup> have a predilection for lodgment in the bones. According to St. George and co-workers,<sup>24d</sup> the necrosis, osteitis and rarefaction of bone in radium poisoning is due to the bombardment of the bones by radium rays. A hypochromic type of anemia is commonly associated with radium<sup>24c</sup> and lead intoxication. These metals<sup>25</sup> conspicuously involve the bones of the jaw, which are especially vulnerable to infection. An occasional case of lead poisoning is seen in which the toxic manifestations of lead seem much out of proportion to the amount of lead taken in, and it is likely that radioactive substances when present are important in enhancing the toxic effect of lead. Inasmuch as commercial lead contains varying amounts of radioactive substances, usually radium D, it likewise should be considered as a possible inciting factor in bronchiogenic carcinoma when such dust and fumes are inhaled over long periods. Although this source possibly does not prevail widely, it conceivably is a definite hazard.

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21. Crawford, B. L.; Stewart, H. L.; Willoughby, C. E., and Smith, F. L.: *Am. J. Cancer* **33**:401, 1938.

22. Aub, J. C.: *J. A. M. A.* **104**:87, 1935.

23. Rajewski, B.: *Radiology* **32**:57, 1939.

24. (a) Stevens, R. H.: *Radiology* **39**:39, 1942. (b) Gettler, A. O., and Norris, C.: *J. A. M. A.* **100**:400, 1933. (c) Martland, H. S.; Colon, P., and Knaf, J. P.: *ibid.* **85**:1769, 1925. (d) St. George, A. V.; Gettler, A. O., and Muller, R. H.: *Arch. Path.* **7**:397, 1929.

25. Aub,<sup>22</sup> Rajewski,<sup>23</sup> Stevens,<sup>24a</sup> Gettler and Norris,<sup>24b</sup> Martland and others,<sup>24c</sup>

## REPORT OF TWO CASES

CASE 1.—A white man aged 57, when admitted to the Edward W. Sparrow Hospital, Lansing, Mich., Jan. 6, 1942, complained of general weakness progressive in character, left foot drop, diplopia, great fatigue on exertion, insomnia and anorexia. He gave a history of having had lead poisoning on two previous occasions, nine and five years ago. Both attacks were associated with intestinal colic and were treated by his physician. The patient had been employed as a linotype operator steadily for thirty-five years. During his work he was exposed to the fumes of molten metal from the linotype machines over a long period. No history of cancer in the family was elicited.

A general examination revealed an overweight white man. There was palsy of the right eye with inability to rotate the eye outward. The other cranial nerves were normal. All of the deep reflexes were slightly increased. The Babinski, Oppenheim and Chaddock signs were negative. The left knee jerk was definitely increased. No ankle clonus was found. The left leg was weaker than the right with a suggestion of a left foot drop. The gums were bluish, and the teeth were carious. The abdomen, the heart and the lungs were essentially normal.

Roentgen examination of the chest revealed an abnormal density between the upper and middle lobes of the right lung. The remainder of the upper lobe of the right lung had a mottled appearance. All other portions of the lung were apparently clear. In addition, a mass was seen extending from the superior mediastinum into the right side of the chest.

The blood on admission contained hemoglobin 81.5 per cent, erythrocytes 4,700,000 and leukocytes 16,000, with 70 per cent neutrophils, 21 per cent lymphocytes, 4 per cent eosinophils and 5 per cent monocytes. No evidence of basophilic stippling was found, but reticulocytes, stippling of erythrocytes and a severe grade of secondary anemia had been observed by the attending physician on several occasions before admission. January 29, the urine revealed 0.15 mg. of lead per thousand cubic centimeters.

The clinical diagnosis was chronic lead poisoning and carcinoma of the lung with metastasis to the mediastinum.

The patient died March 20. The autopsy revealed a neoplasm of the right lung arising in the main bronchus to the middle and upper lobes of the right lung, diagnosed as bronchiogenic carcinoma. Microscopic examination of the tumor of the right lung showed it to be composed of small, polyhedral, deeply staining, poorly differentiated cells. In several areas the neoplasm extended downward from the mucosa of the large bronchus. It extended internal and external to the cartilage plates with metastases to the regional lymph nodes. Gangrenous necrosis of the infarcted area peripheral to the tumor mass of the right lung was seen. Marked general fibrosis and connective tissue hyalinization of the alveolar walls of both lungs were observed. Marked connective tissue hyalinization with calcification was found in the region of the primary site and about some of the metastatic nodules of the right lung. The pleural surfaces showed marked fibrosis and thickening. Acute purulent bronchitis and bronchopneumonia were encountered, which involved chiefly the posterior one third of both lungs. Metastases were found in the peribronchial, peritracheal and mediastinal lymph nodes and in the liver.

CASE 2.—A white man aged 64, when admitted to the Mercy Hospital, Jackson, Mich., Sept. 15, 1941, complained of a productive cough, dyspnea increasing in severity, progressive weakness, night sweats and pain in the left side of the chest. The present illness began about four months prior to admission. The patient had

been in apparently good health up to the present illness and had not consulted a physician. He gave a history of working in an industrial plant as a metal polisher for over ten years in his early career. He worked as a painter during the latter part of his life. No family history of cancer was elicited.

The patient was a well developed and fairly well nourished elderly white man. Diminished breath sounds were present over the left side of the chest, associated with dry and moist rales. The left side of the chest below the inferior angle of the scapula was dull to percussion. A more thorough examination was not permitted because of the grave state of the patient.

The urine showed nothing remarkable. The blood on admission contained hemoglobin 72 per cent, erythrocytes 3,900,000 and leukocytes 17,000, with 86 per cent neutrophils, 12 per cent lymphocytes, 1 per cent eosinophils and 1 per cent monocytes. Nucleated red cells and marked polychromatophilia were seen. The clinical diagnosis was probable carcinoma of the left lung with pleural effusion on the left. No tubercle bacilli were found in either the sputum or the aspirated pleural fluid.

The patient died one week after admission. The autopsy disclosed advanced bronchiogenic carcinoma of the left lung, miliary in type, extensively infiltrating both lungs and pleura. Microscopic examination of the lungs revealed a diffusely infiltrating neoplasm composed of small, irregular shaped, poorly differentiated cells. The primary site appeared to be located in the mucosa and wall of the large bronchus to the upper and lower lobes of the left lung because of the extensive degenerative changes in this region, such as connective tissue hyalinization, cavity formation and calcification. No evidence of tuberculosis was seen. The remainder of the left lung and the entire right lung showed extensive fibrosis of the alveolar walls with well marked connective tissue hyalinization. Extensive fibrous pleuritis of the lungs was encountered, which was extensively infiltrated by the neoplasm. Acute purulent bronchitis and bronchopneumonia were found involving the posterior one third of both lungs.

#### COMMENT

In the 2 cases now presented, exposure to lead continued for many years. An important point, often emphasized, is that carcinoma may not manifest itself until many years after exposure to irritating dusts, which is borne out by these cases. Both the patients were in the typical age group. The first had recurrent attacks of lead intoxication and, in addition, an amount of lead in the urine indicating that lead was actually taken into the body. At autopsy chronic fibroid pneumonitis was found in both cases, suggesting chronic irritation in some form.

Caution should be exercised before concluding that the inhalation of lead has been an inciting factor in bronchiogenic carcinoma. Each case should be carefully studied individually and interpreted absolutely on its own merits. The mere fact that a man worked in a lead industry does not necessarily mean that he has been exposed to the hazards of lead, but a long history of definite exposure to commercial lead dusts and fumes would be significant.

Several cases of lead poisoning have been reported in the establishment where the first patient was employed. In such cases a thorough investigation should be made to determine whether the establishment has

been taking adequate precautions against the hazards of lead. Outbreaks of epidemics of lead poisoning over a long period in the same establishment mean that proper precautions have not been taken. Tests of the atmosphere about the molten pots of lead on the linotype machines where the first patient was employed showed, on one occasion, 16.1 mg. of lead per 10.0 cubic meters of air. Although this sample did not represent the operator's exposure, it did indicate a potential hazard because of an inadequate ventilating system. Moreover, examination with the Geiger counter of three lead slugs from the establishment showed that one slug was slightly radioactive. Although this finding is not important from the standpoint of quantity, it is of interest as it demonstrates a convenient means of checking commercial lead for radioactivity. When tissues are removed, particularly bone, they can be quickly checked for radioactivity by means of the Geiger counter. Regions of the body can also be readily examined for radioactivity by this instrument. Evans<sup>26</sup> described a method in the use of the Geiger counter which is of great practical importance in medicine. By measuring the amount of gamma radiation, he can estimate the amount of radium present. When only traces of radioactive substances are present, it may be necessary to do a quantitative chemical analysis, because small amounts may not be detected by this instrument.

#### SUMMARY

Carcinoma of the lung in lead workers has been recorded in the literature. The Schneeberg cases are the only conclusive group of cases of bronchiogenic carcinoma associated with the inhalation of radioactive substances. Because commercial lead is by far the most radioactive of all the common metals, owing to the presence of radioactive elements, particularly radium D, it may be a possible factor in the production of bronchiogenic carcinoma when lead dusts and lead fumes are inhaled.

Two cases of bronchiogenic carcinoma are reported. Both patients gave a long history of occupational contact with commercial lead. The first gave a protracted history of exposure and intoxication. Both showed chronic fibroid pneumonitis.

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26. Evans, R. D.: *Am. J. Roentgenol.* **37**:368, 1937.

# MORPHOLOGIC CHANGES IN THE RAT'S ADRENAL CORTEX UNDER VARIOUS EXPERIMENTAL CONDITIONS

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It is well recognized today that disturbances in electrolyte, carbohydrate and protein metabolism influence the activity of the adrenal gland. Considerable information is available concerning the interdependence between the adrenal and the other endocrine glands, particularly the gonads and the pituitary, increased and decreased function of the latter profoundly affecting adrenal function. This study, undertaken with the view first expressed by Virchow that every physiologic process has its anatomic counterpart, is concerned with the morphologic changes in the rat's adrenal cortex associated with various endocrine and metabolic disturbances. These include hypophysectomy, castration, variations in the potassium and the protein content of the diet, inanition and administration of desoxycorticosterone acetate and stilbestrol. Knowledge of the life cycle of the cortical cell (Zwemer<sup>1</sup>) in its migration from the zona glomerulosa through the zona fasciculata to die in the zona reticularis and the more recently available histochemical methods which identify the cortical steroid hormones and cholesterol respectively (Bennett<sup>2</sup>) lend added significance to the histologic changes and alterations in lipid pattern observed in the adrenal cortex under various experimental conditions, thereby opening the way to physiologic interpretations.

## MATERIALS AND METHODS

The adrenals examined were obtained in large part from rats being studied for other purposes by various investigators working in the laboratories of the Yale School of Medicine. Drs. D. C. Darrow and S. H. Durlacher supplied rats maintained on a low potassium diet and others given desoxycorticosterone acetate; Dr. J. A. Russell performed the hypophysectomies; Drs. F. Engel and J. Tepperman provided rats maintained on a high protein diet, and Miss Edith Fry, stilbestrol-treated rats. Male and female rats of Wistar, Yale and Sprague-Dawley strains, weighing approximately 200 Gm., were studied. The adrenals with the surrounding fat were carefully removed at autopsy and immediately placed in 4 per cent solution of formaldehyde. After two days, following the removal of the periadrenal tissues, the glands were dried on paper toweling and weighed

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This study was aided by a grant from the Commonwealth Fund.

From the Laboratory of Pathology of the Yale University School of Medicine

1. Zwemer, R. L.: *Am. J. Path.* **12**:107, 1936.

2. Bennett, H. S.: *Am. J. Anat.* **67**:151, 1940.



on a chainomatic balance to the nearest milligram. The weights of the glands are always expressed as grams per kilogram of body weight. Paraffin sections were stained with hematoxylin and eosin, and frozen sections, cut at 10 to 15 microns, were stained with sudan IV. The phenylhydrazine and digitonin techniques (as employed by Bennett<sup>2</sup>) were used on selected adrenals to demonstrate corticosteroids and cholesterol, respectively.

#### THE NORMAL ADRENAL CORTEX

To determine the significance of an adrenal weight one must necessarily consider the weight of the animal. Furthermore, in those experimental conditions in which the animal loses weight, the ratio of the adrenal weight to the original body weight as well as to the final body weight should be considered. False conclusions are easily drawn from absolute adrenal weights if the ratio of each of the latter to the body weight is not taken into account. Using rats from the Yale and Wistar strains, Cole and Harned<sup>3</sup> found straight line relationships between adrenal and body weights in males and females whose body weight varied from 100 to 320 Gm. That the female adrenal is heavier is well known. Andersen and Kennedy<sup>4</sup> showed that the adrenals of females are slightly larger in estrus. The weights of the adrenals of the normal male and female rats of the Wistar strain are shown in table 1.

Microscopic examination of the normal rat adrenal reveals the zona glomerulosa to be several cells thick and laden with lipoid (fig. 1). A thin band of fat-free polygonal cells comprises the junction of the zona glomerulosa and the zona fasciculata ("clear zone"). The cells of the zona fasciculata exhibit an orderly columnar arrangement. Their cytoplasm is foamy and contains abundant lipoid in the form of uniformly moderate-sized droplets. The outer part of the zona fasciculata is much richer in lipoid than the inner. The former corresponds to Bennett's "secretory layer," while the latter is the "discharging layer." The zona reticularis in the normal rat adrenal is poorly defined, consisting of a narrow band of cells in various stages of degeneration. No histologic sex differences can be ascertained aside from the wider cortex in the female.

Employing phenylhydrazine and digitonin on frozen sections, one sees that the cortical steroids and the cholesterol are most concentrated in the outer half of the zona fasciculata with somewhat less in the zona glomerulosa. In general it can be said from histochemical studies of adrenals of rats as well as of those of man and dogs<sup>5</sup> that the

3. Cole, V. V., and Harned, B. K.: *Endocrinology* **30**:146, 1942.

4. Andersen, D. H., and Kennedy, H. S.: *J. Physiol.* **76**:247, 1932.

5. Sarason, E. L.: (a) *A Morphological Study of the Adrenal Cortex in Systemic Disease*, to be published; (b) unpublished data.

distribution of the ketosteroids and of the cholesterol corresponds closely to the intensity of the sudanophilic material. For this reason histochemical studies were not employed routinely in the present study, as it was believed that the all-inclusive sudan stain served the purpose adequately.

#### DESOXYCORTICOSTERONE ACETATE

Crystalline desoxycorticosterone acetate (abbreviated in tables to DOCA)<sup>6</sup> was dissolved in warm 95 per cent alcohol and added to physiologic solution of sodium chloride so that each cubic centimeter contained 2 mg. of finely suspended precipitate in 7 per cent alcohol. Two milligrams of the drug was administered subcutaneously daily for one month to 19 male and 10 female rats of the Wistar strain. The weights of the glands as compared with normal weights are presented in table 1.

TABLE 1.—*Effect of Desoxycorticosterone on the Weight of the Rat Adrenals*

Group	Sex	Rats *	Adrenal Weight, Gm. per Kg.	p †
Normal.....	M	8	0.1449 $\pm$ 0.0049	
DOCA-treated.....	M	19	0.0997 $\pm$ 0.0034	<0.01
Normal.....	F	20	0.2382 $\pm$ 0.00527	
DOCA-treated.....	F	10	0.2260 $\pm$ 0.0051	>0.05

\* The rats were given 2 mg. of desoxyeorticosterone acetate daily for one month.

† According to R. A. Fisher (Statistical Methods for Research Workers, London, Oliver & Boyd, 1941, pp. 120-125), a value of *p* greater than (>) 0.05 indicates no significant difference between the means; a value of *p* less than (<) 0.05 but greater than 0.01 indicates a significant difference; a value of *p* less than 0.01 indicates a highly significant difference.

Following daily administration of 2 mg. of desoxycorticosterone acetate for one month, there occurred considerable atrophy of the male adrenal but no significant change in the female adrenal. One is struck by the variance of results reported in the literature concerning the effects of cortical hormones. Wells and Kendall<sup>7</sup> reported adrenal atrophy occurring eight days after administration of corticosterone, but no atrophy after 10 mg. of desoxycorticosterone suspended in 0.7 per cent solution of sodium chloride had been injected subcutaneously every third day for nine days. Selye and Dosne<sup>8</sup> observed slight cortical atrophy following daily administration of 2 mg. of desoxycorticosterone acetate for twenty days, and more pronounced atrophy after 10 mg. had been given daily for a similar period. Selye<sup>9</sup> stated that the atrophy follow-

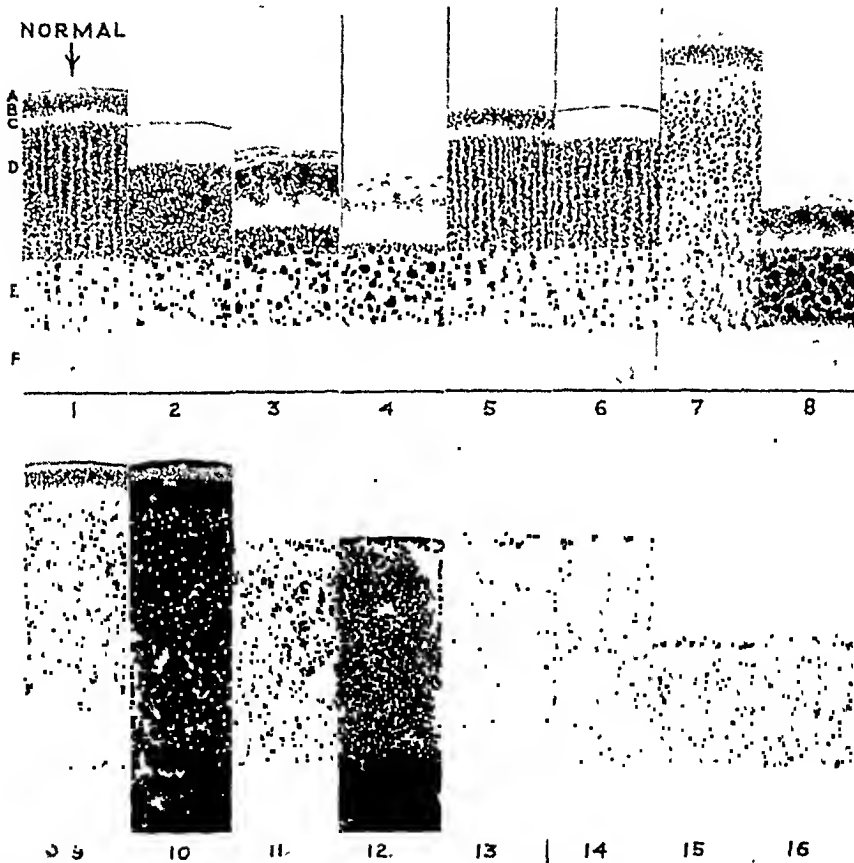
6. The crystalline desoxycorticosterone was generously supplied by the Ciba Company.

7. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**: 133, 1940.

8. Selye, H., and Dosne, C.: Proc. Soc. Exper. Biol. & Med. **44**:165, 1940.

9. Selye, H.: Canad. M. A. J. **42**:113, 1940.

ing administration of this compound is more pronounced in the female. As may be seen from table 1, the comparatively small dose of 2 mg. of desoxycorticosterone acetate causes significant atrophy only in the male. Carnes and his co-workers<sup>10</sup> observed more striking atrophy in the male than in the female following daily administration of 5 mg. of this substance for one month. Ingle<sup>11</sup> found it necessary to administer larger amounts of an extract of adrenal cortex to females than to males in order to produce a loss in the weight of the adrenal glands. It is known that castration of the female may result in atrophy of the



(See legend on opposite page)

adrenal glands. Administration of estrogen causes hypertrophy of the gland<sup>12</sup> while that of testosterone has the opposite effect. It would be expected, therefore, that the female adrenal would be more resistant to atrophy.

Ingle and Kendall<sup>13</sup> found that the administration of large amounts of extract of adrenal cortex over a period of seven days caused atrophy

10. Carnes, W. H.; Ragan, C.; Ferrebee, J. W., and O'Neill, J.: *Endocrinology* **29**:144, 1941.

11. Ingle, D. J.: *Endocrinology* **24**:194, 1939.

12. Bourne, G., and Zuckerman, S.: *J. Endocrinol.* **2**:283, 1941.

13. Ingle, D. J., and Kendall, E. C.: *Science* **86**:245, 1937.

Fig. 1.—Normal rat adrenal. The black dots represent sudanophilic material. *A* indicates thin acellular fat-free capsule; *B*, zona glomerulosa; *C*, "clear zone" of the outer part of the zona fasciculata; *D*, outer part of the zona fasciculata; *E*, inner part of the zona fasciculata; *F*, medulla.

Fig. 2.—Rat B1, treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the atrophy of the cortex and the depletion of lipid in the zona glomerulosa.

Fig. 3.—Rat H16, a hypophysectomized rat maintained on a normal diet (one month after hypophysectomy). Note the thickened cellular capsule with a few lipid-containing cells, the widened zona glomerulosa, rich in lipid, and the prominent clear zone. The sudanophilic material of the shrunken zone fasciculata is in the form of large droplets.

Fig. 4.—Rat H12, a hypophysectomized rat treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the further atrophy of the cortex with depletion of lipid of the zona glomerulosa.

Fig. 5.—Rat B6, an ovariectomized rat. Note the relatively normal appearance of the cortex.

Fig. 6.—Rat B11, an ovariectomized rat treated with 2 mg. of desoxycorticosterone acetate daily for one month. Note the zona glomerulosa depleted of lipid.

Fig. 7.—Rat S18, a female, starved for seven days. Note the hypertrophy of the cortex and the general depletion of lipid. Pseudoacini are seen in the inner part of the zona fasciculata.

Fig. 8.—Rat H3, a hypophysectomized animal, suffering from chronic inanition for one month. Note the striking atrophy of the inner part of the zona fasciculata with the lipid present in the form of unusually large droplets.

Fig. 9.—Rat HP1, a male fed a high protein-low carbohydrate diet for one month. Note the hypertrophy of the cortex with depletion of the lipid of the inner part of the cortex. Compare with the control (fig. 11).

Fig. 10.—Rat HP1, same as in figure 9. Frozen section treated with digitonin and examined with the polariscope. The white dots represent birefringent cholesterol crystals. Note the general depletion of the latter in the zona fasciculata with the increased amount in the zona glomerulosa. Compare with the control (fig. 12).

Fig. 11.—Rat CT1—Litter mate of rat HP1 fed the normal chow diet. Contrast with figure 9.

Fig. 12.—Rat CT1.—This is a digitonin preparation of an adrenal of the animal fed the chow diet. Note the general distribution of the birefringent cholesterol crystals through the entire cortex. Compare with figure 10.

Fig. 13.—Rat ST2, a starved male rat given an injection of stilbestrol. Note the general depletion of lipid associated with the presence of vacuolated non-lipoid-containing cells in the outer part of the zona fasciculata. Compare with figure 14.

Fig. 14.—Rat C2, a male rat starved for a period similar to rat ST2 but not treated with stilbestrol. Note the relatively normal-appearing cortex as compared with figure 13.

Fig. 15.—Rat SH2, a hypophysectomized, starved rat treated with stilbestrol. The animal was put to death fifty-four hours after hypophysectomy. Note the atrophy of the cortex with but little change in the lipid pattern.

Fig. 16.—Rat FH4, a hypophysectomized starved rat not treated with stilbestrol. Note that the cortex appears similar to that of the stilbestrol-treated hypophysectomized animal (fig. 15).

of the adrenals of male rats. Ingle, Higgins and Kendall later reported <sup>14a</sup> that compound A and corticosterone also produced adrenal atrophy. Ingle <sup>14b</sup> demonstrated that simultaneous administration of an adrenotropic fraction of the pituitary gland prevents the atrophy of the adrenal that ordinarily follows treatment with extract of adrenal cortex alone. It was therefore suggested by Ingle that the adrenotropic activity of the pituitary gland is suppressed by excessive extract of adrenal cortex and that in this manner adrenal atrophy is produced. It is difficult to subscribe to this hypothesis in view of the radically different histologic appearance of the adrenal after hypophysectomy and administration of cortical extract (figs. 2 and 3). There was observed in the rats (both males and females) treated with desoxycorticosterone acetate a reduction in the size of the zona glomerulosa associated with a disappearance of the sudanophilic lipid, cholesterol and cortical steroids. Similar depletion of the lipoids was found by Flexner and Grollman <sup>15</sup> (employing the osmic acid technic) after administration of extract of adrenal cortex and by Carnes and associates, <sup>10</sup> who treated rats with desoxycorticosterone acetate.

The depletion in the glomerulosa cell lipid following administration of excess extract of adrenal cortex was the basis in part for the statement of Flexner and Grollman <sup>15</sup> that depression of adrenal activity is associated with loss of cortical lipid. Dosne and Dalton <sup>16</sup> and Selye <sup>17</sup> took issue with this conclusion. They expressed the belief that the amount of cortical lipid demonstrated by either sudan stain or osmic acid definitely decreases as the adrenal enlarges with increased activity. This view is borne out in my experience.<sup>5</sup> I have observed in human cases of overwhelming infection and cachexia markedly enlarged adrenals depleted of lipid. Severe depletion has been noted in the adrenals of rats exposed to high altitudes <sup>18</sup>—conditions which have been shown by Evans <sup>19</sup> and Thorn and co-workers <sup>20</sup> definitely to activate these glands. It would appear, therefore, that the stimulated adrenal cortex often becomes depleted of lipid. This might be interpreted as evidence of rapid discharge and minimal storage of cortical hormone during periods of heightened adrenal activity in an

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14. (a) Ingle, D. J.; Higgins, J. M., and Kendall, E. C.: *Anat. Rec.* **71**:363, 1938. (b) Ingle, D. J.: *Am. J. Physiol.* **124**:369, 1938.

15. Flexner, L. B., and Grollman, A.: *Anat. Rec.* **75**:207, 1939.

16. Dosne, C., and Dalton, A. J.: *Anat. Rec.* **80**:211, 1941.

17. Selye, H.: *Endocrinology* **21**:169, 1937.

18. Sarason, E. L.: To be published.

19. Evans, G. T.: (a) *J. Biol. Chem.* **105**:34, 1934; (b) *Am. J. Physiol.* **114**:297, 1936.

20. Lewis, R. A.; Thorn, G. W.; Koepf, G. F., and Dorrance, S. D.: *J. Clin. Investigation* **21**:33, 1942.

effort to meet the demands of the body for increased amounts of the vital hormone. It cannot be denied that in general an endocrine organ is rendered inactive by administration of excessive amounts of its hormone. Such must be the case with the adrenal glands after administration of desoxycorticosterone. It must be borne in mind, therefore, that under certain conditions the absence of cortical lipid may be associated with either activity or rest of the gland.

#### VARIATIONS OF POTASSIUM IN THE DIET

Male and female rats of Sprague-Dawley and Wistar strains were maintained for one month on (a) a diet low in potassium, (b) a low potassium diet plus drinking water containing potassium chloride in the concentration of 1.5 per cent and (c) regular Purina fox chow plus the 1.5 per cent potassium chloride drinking water. The latter combination represented the high potassium diet. The synthetic low potas-

TABLE 2.—*Effect of Variations of the Potassium Content of the Diet on the Weight of Rat Adrenals*

Diet	Sex	Rats	Adrenal Weight, Gm. per Kg.	<i>p</i>
Purina chow.....	M	8	0.1449 $\pm$ 0.0049	
Chow + KCl....	M	5	0.1559 $\pm$ 0.0085	>0.05
Low K.....	M	10	0.1328 $\pm$ 0.0028	
Low K + KCl.....	M	8	0.1382 $\pm$ 0.0061	>0.05
Low K.....	F	4	0.2560 $\pm$ 0.0187	
Chow + KCl.....	F	5	0.2688 $\pm$ 0.0121	>0.05

sium diet as used by Durlacher, Darrow and Winternitz<sup>21</sup> contained 1.6 millimols of potassium per hundred grams, while the potassium content of the Purina chow was 15.5 millimols per hundred grams and that of the synthetic diet with added potassium chloride 16.6 millimols per hundred grams. The adrenal weights are presented in table 2.

From the data in table 2 it is seen that the adrenals of these rats exhibited no significant change in weight whether the animals were fed a diet low or a diet rich in potassium. Microscopic examination of these glands revealed no apparent alteration in the lipid pattern of the cortex. Ingle and Kendall<sup>22</sup> likewise observed no change in the size of the adrenal glands of animals fed different amounts of sodium and potassium.

The experiments described shed light on the nature of the effect of desoxycorticosterone acetate on the adrenals. Animals fed a low potassium diet or given injections of this compound exhibit a similar

21. Durlacher, S. H.; Darrow, D. C., and Winternitz, M. C.: *Am. J. Physiol.* **136**:346, 1942.

22. Ingle, D. J., and Kendall, E. G.: *Am. J. Physiol.* **122**:585, 1938.

loss of muscle potassium accompanied by low serum potassium values (Heppel<sup>23</sup>; Miller and Darrow<sup>24</sup>). Whereas desoxycorticosterone acetate (2 mg. daily for one month) produced atrophy of the adrenal, this was not observed in animals fed a low potassium diet. Furthermore, the shrinkage of the cells of the zona glomerulosa associated with the depletion of lipid seen following administration of the drug was not observed in the low potassium group. It is well recognized that the adrenal and the kidney are intimately concerned with potassium metabolism. It appears that whereas renal hypertrophy results from lowering of the muscle and serum potassium by either dietary methods or by administration of desoxycorticosterone acetate,<sup>21</sup> the adrenal atrophy

TABLE 3.—*Effect of Hypophysectomy on the Weight of the Adrenals of the Female Rat*

Group	Rats	Adrenal Weight, Gm. per Kg. of Initial Body Weight	p
Normal.....	20	0.2382 $\pm$ 0.0052	
Hypophysectomized.....	4	0.0929 $\pm$ 0.0028	<0.01

TABLE 4.—*Effect of a Low Potassium Diet, of Desoxycorticosterone and of Chronic Inanition on the Weight of the Adrenals of the Hypophysectomized Female Rat*

Group	Rats	Adrenal Weight, Gm. per Kg. of Initial Body Weight	p
Normal diet.....	4	0.0929 $\pm$ 0.0028	
Low K diet.....	4	0.1024 $\pm$ 0.0030	>0.05
DOCA.....	5	0.0797 $\pm$ 0.0035	<0.05; >0.01
Inanition.....	3	0.0551 $\pm$ 0.0056	<0.01

produced by the latter is not dependent directly on a deficiency of body potassium.

#### HYPOPHYSECTOMY

The adrenals of 16 female and 4 male rats were examined one month and two days, respectively, after hypophysectomy. Some of the hypophysectomized animals were maintained on a low potassium diet, and others were given 2 mg. of desoxycorticosterone acetate daily during the post-operative month. The weights of the adrenal glands are presented in tables 3 and 4.

In hypophysectomized female rats maintained on a normal diet a considerable degree of atrophy of the adrenal cortex was found one month after operation. The usual acellular capsule had been trans-

23. Heppel, L. A.: *Am. J. Physiol.* **127**:385, 1939.

24. Miller, H. C., and Darrow, D. C.: *Am. J. Physiol.* **132**:801, 1941.

formed into a band rich in oval-shaped cells, some of which contained sudanophilic material (fig. 3). The zona glomerulosa was intact, if not a little wider than usual, and contained abundant lipoid. Digitonin preparations revealed that the sudanophilic zona glomerulosa was laden with cholesterol. The fat-free clear zone of the outer part of the zona fasciculata was very prominent. The cells comprising the outer part of this zone lacked their usual regular columnar arrangement, and their cytoplasm appeared vacuolated instead of foamy. The sudan stain of these cells was in the form of large droplets of varying size, rather than the usual uniformly small droplets. The inner part of the zona fasciculata was sparse in cells, and their cytoplasm contained abundant yellow-green pigment. Pyknotic and extruded nuclei were frequent in the zona reticularis, as well as marked congestion and occasional hemorrhage.

The adrenals of hypophysectomized rats fed a low potassium diet differed neither grossly nor microscopically from those of hypophysectomized animals given the regular diet. Further significant reduction in adrenal size was seen when the hypophysectomy was followed by the administration of desoxycorticosterone acetate (2 mg. daily for thirty days). Histologically, as in the intact animal, the adrenals of the desoxycorticosterone-treated hypophysectomized rat exhibited marked depletion of the lipoid of the zona glomerulosa (fig. 4).

The striking adrenal atrophy of hypophysectomized rats suffering from chronic inanition will be discussed in later paragraphs in connection with the general problem of inanition.

The adrenals of 4 hypophysectomized male rats (Yale strain) that had been subjected to a fifty-four hour postoperative fast were found to have already undergone atrophy (table 8). (Two of these rats had received a single injection of stilbestrol. As in the intact animal, in the hypophysectomized animal the stilbestrol did not affect the size of the adrenals.) Although atrophy had resulted in so short a time following hypophysectomy, the histologic structure was not perceptibly altered except for shrinkage of the cortex (fig. 16).

In 1930 Smith<sup>25</sup> reported marked adrenal atrophy following ablation of the pituitary, with restoration of the adrenals to normal size following replacement therapy. Cutuly<sup>26</sup> has shown conclusively that the atrophy is exclusively cortical. Smith noted the retrogression of the cortex to be rapid, the adrenals in 1 animal killed six days after hypophysectomy losing half of their weight. From the data in table 8 it is seen that after hypophysectomy followed by a fifty-four hour fast the adrenals are decreased one third in weight. Crooke and Gilmour<sup>27</sup>

25. Smith, P. E.: *Am. J. Anat.* **45**:205, 1930.

26. Cutuly, E.: *Anat. Rec.* **66**:119, 1936.

27. Crooke, A. C., and Gilmour, J. R.: *J. Path. & Bact.* **47**:525, 1938.



reported a decrease in adrenal weight from a normal value of about 19 mg. to 15 mg. and 12 mg., two and four days, respectively, after hypophysectomy. Perla<sup>28</sup> described degenerative changes in the zona reticularis as early as four days after hypophysectomy. Crooke and Gilmour<sup>27</sup> likewise found that loss of cortical cells and degenerative changes did not appear until four days after hypophysectomy. They observed that the cortical atrophy was confined to the inner zone, the outer cortical zone being increased in depth. Some of the pigment present in the inner cortical cells was shown by these authors to be iron positive, the remainder apparently being lipofuscin. These findings are confirmed in the present study.

Whereas Smith<sup>25</sup> observed abundant lipid in the outer cortex with depletion in the inner cortex, the histologic picture seen in the present study can best be described as a "bull's eye"—the widened zona glomerulosa and the atrophied zona fasciculata filled with lipid separated by a wide clear zone free of fat. A morphologic interpretation of the histologic changes in the cortex following hypophysectomy is possible on the basis of the theory (Zwemer<sup>1</sup>; Bennett<sup>2</sup>) postulating that the cortical cells originate from the capsule and migrate through the cortex to die in its inner portion; the proliferation of primitive capsular cells (some containing lipid) and of young cells of the zona glomerulosa may be an attempt to replace the aging inner cortical cells which suffer premature death following hypophysectomy. Leblond and Nelson<sup>29</sup> observed persistence of lipid in the atrophic inner part of the cortex following hypophysectomy. In view of the work of Selye<sup>17</sup> and of Houssay and associates<sup>30</sup> showing that very fine powder-like sudanophilic material is indicative of adrenal activation, it should be pointed out that the atrophic inactive inner portion of the cortex is the site of large droplets of sudan. Whereas after hypophysectomy the derangement of electrolyte and water metabolism is only slight, as compared with the profound disturbances in carbohydrate metabolism, it has been stated by Swann<sup>31</sup> that "presumably, the portions of the cortex only slightly affected by hypophysectomy, i. e., primarily the glomerulosa layer, secrete the 'salt and water' hormone. . . . Since hypophysectomy leads to degeneration of the internal layers of the cortex, it seems plausible to ascribe to them the adrenal secretions not produced after hypophysectomy, i. e., the steroids responsible for the effects on sugar metabolism."

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28. Perla, D.: *Proc. Soc. Exper. Biol. & Med.* **32**:655, 1935.

29. Leblond, C. P., and Nelson, W. V.: *Compt. rend. Soc. de biol.* **124**:9, 1937.

30. Houssay, B. A.; Busotti, H.; Mazzacco, P., and Sammartino, R.: *Compt. rend. Soc. de biol.* **144**:739, 1933.

31. Swann, H. G.: *Physiol. Rev.* **20**:493, 1940.

Ingle and co-workers<sup>14a</sup> stated: "The extent of atrophy of the glands of animals treated with cortin resembled that which occurs in totally hypophysectomized animals." Because the administration of a pituitary extract containing the adrenotropic hormone prevents the adrenal atrophy resulting from the administration of extract of adrenal cortex, it has been argued by Ingle<sup>14b</sup> that the atrophy is the result of pituitary depression following the administration of the extract of adrenal cortex. Were this so, one would expect the histologic changes in the adrenal following hypophysectomy to be similar to those following administration of desoxycorticosterone acetate. This is not the case. Whereas the adrenal of the rat treated with desoxycorticosterone acetate has a lipid-poor zona glomerulosa and a normal-appearing zona fasciculata, the gland of the hypophysectomized animal has a widened zona glomerulosa, abundant in lipid, associated with a zona fasciculata showing marked shrinkage. The even more marked adrenal atrophy and

TABLE 5.—*Effect of Acute Inanition on the Weight of Rat Adrenals*

Starvation Period	Sex	Rats	Loss of Body Weight	Adrenal Weight		p
				Gm. per Kg. Initial Body Weight	Gm. per Kg. Final Body Weight	
Fed controls.....	M	8	0	0.1449 ± 0.0049		
One day.....	M	3	12%	0.1511 ± 0.0051	0.1716	>0.05
Two days.....	M	3	15%	0.1640 ± 0.0042	0.1939	<0.05; >0.01
Seven days.....	M	6	25-30%	0.1576 ± 0.0038	0.2235	<0.05; >0.01
Fed controls.....	F	20	0	0.2383 ± 0.0052		
Seven days.....	F	3	25-28%	0.2399 ± 0.0351	0.3966	<0.01

depletion of lipid of the zonâ glomerulosa of the hypophysectomized rat treated with desoxycorticosterone acetate as compared with the treated intact animal is more evidence against the theory that the atrophy following administration of desoxycorticosterone acetate is mediated through the pituitary.

#### INANITION

Male and female rats of the Wistar strain were subjected to acute inanition for a period of one to seven days, during which time they received drinking water but not food. The weights of the adrenal glands are presented in table 5.

During acute inanition the adrenals, in contrast to the other viscera, lost no weight; on the contrary, they gained a little and thereby increased their relative and absolute percentage weights. The data in table 5 confirm the findings of Jackson,<sup>32</sup> who observed that the adrenals of male rats during acute inanition gained 1.5 per cent in weight. This increase was not regarded by Jackson as significant. A statistical analy-

32. Jackson, C. M.: *Am. J. Anat.* 25:221, 1919.

sis of the data in table 5 reveals that the slight increase in the adrenal weight of male rats subjected to one day of starvation is not significant, while the increases seen following two and seven days' starvation are significant. Even more significant adrenal hypertrophy was observed in females starved seven days. This sex difference is apparent from the data of Mulinos and Pomerantz<sup>33</sup> if the original body weights of the animals are taken into account. From their table it appears that the average adrenal weight of the male rats increased from 28 to 34 mg. after seven days of starvation. They stated that the starved animals weighed 277 Gm. before starvation, in contrast to the fully fed controls, weighing 200 Gm. A definite linear relationship between adrenal and body weight is known to exist. Hence, if the difference between the initial weights of the starved animals and those of the controls is taken into account, one cannot admit that any significant hypertrophy of the male adrenals has occurred. These authors observed an increase in the weight of the female adrenals during acute inanition comparable to that noted in table 5.

Microscopic examination reveals no constant lipid pattern of the adrenals during acute inanition. The glands of about half the animals that had fasted for seven days showed varying degrees of depletion of the lipid of the zona fasciculata, with sudanophilic material persisting in the zona glomerulosa (fig. 7). A more uniform distribution of fine powder-like sudanophilic material was noted throughout the cortex in the other animals, the clear zone and the inner part of the zona fasciculata possessing more lipid than usual. Similarly varying patterns of lipid distribution were described by Jackson.<sup>32</sup> Whitehead<sup>34a</sup> recently reported a striking species difference between guinea pigs and rabbits during acute inanition. Whereas the adrenals of fasting rabbits show no alteration in the amount or the distribution of cortical fat, the glands of guinea pigs show a varying lipid pattern depending on the duration of the inanition. In general it appears that Whitehead's observations on the guinea pig correspond closely to the findings presented in this study, i. e., increase of inner cortical lipid during the first few days of starvation with a tendency toward depletion of fat after a week or more of inanition. Dosne and Dalton<sup>16</sup> observed some reduction of the lipid content of the cortex in the adrenals of rats starved twenty-four hours. The explanation of the variation in lipid pattern seen in acute inanition is not at hand. The slight increase in cortical lipid pattern seen after one or two days of fasting may represent increased production of hormone, while the cortical depletion seen in

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33. Mulinos, M. G., and Pomerantz, L.: *Am. J. Physiol.* **132**:368, 1941.

34. Whitehead, R. J.: (a) *J. Path. & Bact.* **54**:169, 1942; (b) *Brit. J. Exper. Path.* **13**:200, 1932.

some animals following more prolonged starvation may be the result of hormone discharge. Formation of pseudoacini at the corticomedullary junction was seen in the lipoid-depleted adrenals of 2 female rats (fig. 7).

Mention should be made of a group of 10 hypophysectomized females which did very poorly postoperatively, 7 dying in the course of the month (the adrenals were not examined). The 3 survivors lost approximately 50 per cent of their body weights during the month. Their food consumption during this period was definitely decreased. The explanation of their slowly progressive decline is not at hand. As may be seen from table 4, the most severe adrenal atrophy, associated with extreme reduction of the inner portion of the cortex, was exhibited by this group of 3 animals (fig. 8). Mulinos and Pomerantz<sup>33</sup> observed that normal pituitary glands implanted into chronically underfed female rats resulted in a gain of weight in the otherwise atrophied adrenal glands. These authors reasoned (after the manner of Ingle<sup>14b</sup>) that the

TABLE 6.—*Effect of a High Protein Diet on the Weight of the Rat Adrenals*

Diet	Sex	Rats	Adrenal Weight, Gm. per Kg.	<i>p</i>
Purina chow.....	M	18	0.157 ± 0.0087	
High protein.....	M	18	0.236 ± 0.0115	
				<0.01

adrenal atrophy seen in malnutrition was due in part to the insufficiency of adrenotropic hormone resulting from the physiologic depression of function of the pituitary. The aforementioned data seem to indicate that factors other than depression of the function of the pituitary may effect atrophy of the adrenal in malnutrition.

#### HIGH PROTEIN DIET

Yale strain rats were fed a diet consisting exclusively of ground lean meat, with a protein content of approximately 45 to 50 per cent, for one month. Other rats of the same strain were fed the basic diet including the fox chow, containing 18 per cent protein. The adrenal weights of these animals are presented in table 6.

The marked increase in size of the adrenals of rats fed a high protein diet is striking. Microscopic examination revealed the hypertrophy to be cortical (fig. 9). The zona glomerulosa was slightly widened by virtue of an increased amount of lipoid in the cells. The cells of the zona fasciculata lacked their usual foamy appearance for the most part, taking on a homogeneously eosinophilic color (hemotoxylin-eosin preparation). They were moderately depleted in lipoid. The clear zone, composed of the outermost fat-free cells of the zona fasciculata, was

increased in width. The digitonin preparations revealed that the zona glomerulosa of the hypertrophied adrenal contained an increased amount of cholesterol and that the zona fasciculata was markedly depleted of birefringent material (fig. 10). The distribution of the latter differs markedly from the uniform distribution of this material through both the zona glomerulosa and the zona fasciculata of the normal adrenal (fig. 12).

Farr<sup>35</sup> described marked adrenal hypertrophy of rabbits which had been fed a diet consisting exclusively of milk and eggs. He noted that the zona fasciculata was widened and the cells vacuolated. Whitehead<sup>34b</sup> reported cortical proliferation in the mouse adrenal after administration of peptone. He stated: "They [the mitoses in the cortex] indicate the response to demand for increased function." Engel and Tepperman,<sup>36</sup> who have studied the metabolism of such meat-fed rats, expressed the belief that the observed adrenal hypertrophy is associated with an increase in the rate of glyconeogenesis, the latter being a consequence of a high protein-low carbohydrate diet. These authors have proposed in a review of adrenal hypertrophy<sup>37</sup> that accelerated protein catabolism is the common denominator of many conditions associated with adrenal hypertrophy. It should be pointed out that the enlarged stimulated adrenals are depleted of lipoid, confirming the view that in general activation of the adrenals is associated with depletion of cortical lipoid. In foregoing paragraphs similar degrees of depletion have been described in association with adrenal hypertrophy following starvation. The same association has been noted by Dosne and Dalton.<sup>16</sup> Enlarged human adrenals depleted of lipoid have been seen in cases of rapidly advancing cachexia associated with cancer.<sup>5a</sup> James and Nelson<sup>38</sup> have reported adrenal hypertrophy in association with increased glyconeogenesis following administration of stilbestrol. Loeser<sup>39</sup> observed enlargement of the glands and loss of lipoid following daily administration of 1 mg. of stilbestrol for three weeks. The adrenals of several rats included in the present study given a single injection of stilbestrol were found to be partially depleted of lipoid although not changed in size (see a later paragraph). From the foregoing data and the work cited it appears that adrenal hypertrophy and depletion of cortical lipoid are characteristic of accelerated protein catabolism as seen in animals fed a high protein diet, subjected to fasting or treated by injection of stilbestrol.

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35. Farr, T. H.: *Verhandl. d. deutsch. path. Gesellsch.* **15**:234, 1912.

36. Engel, F., and Tepperman, S. J.: Unpublished data.

37. Engel, F., and Tepperman, S. J.: *Metabolic Determinants of Adrenal Size and Function*, to be published.

38. James, R. G., and Nelson, W. O.: *Am. J. Physiol.* **136**:136, 1942.

39. Loeser, A.: *Ztschr. f. d. ges. exper. Med.* **105**:430, 1939.

## OVARIECTOMY

The adrenals and kidneys of 6 ovariectomized 10 week old Wistar rats were examined one month after operation. Three animals of this group received 2 mg. of desoxycorticosterone acetate daily during the postoperative period. The wet and dry weights of the kidneys were determined. The results are presented in table 7.

Considerable controversy has appeared in the literature (Hashimoto<sup>40</sup>) concerning the effect of ovariectomy on the adrenals. The age of the rat at the time of operation and the length of the postoperative survival are important factors determining the size of the adrenals. In the present series ovariectomy resulted in no significant atrophy of the adrenals one month after operation. Andersen and Kennedy,<sup>41</sup> working with animals of similar age, reported divergent results. Histologi-

TABLE 7.—*Effect of Ovariectomy and Administration of Desoxycorticosterone Acetate on the Weight of the Adrenals and the Weight of the Kidneys in Castrated Female Rats*

Group	Rats	Adrenal Weight, Gm. per Kg.	p	Kidney Weight, Gm. per Kg.	
				Wet	Dry
Normal females.....	20	0.2382 $\pm$ 0.0052	>0.5	7.62	1.77
Ovariectomized.....	3	0.2096 $\pm$ 0.0285		7.02	1.60
Ovariectomized and treated with DOCA.....	3	0.1990 $\pm$ 0.0156	>0.5	9.55	2.05
DOCA-treated normal.....	10	0.2260 $\pm$ 0.0051		9.07*	2.14*

\* These weights were reported by Durlacher, Darrow and Winternitz,<sup>21</sup> who have described renal hypertrophy following administration of desoxycorticosterone.

cally, the adrenals of the ovariectomized animals exhibited no alteration in lipid pattern as compared with the normal (fig. 5).

In view of the resistance of the adrenals of normal female rats to atrophy following the daily administration of 2 mg. of desoxycorticosterone acetate for one month, this compound was given in similar doses to 3 ovariectomized rats. As may be seen in table 7, the adrenals of the animals so treated were not significantly smaller than those of untreated ovariectomized animals although, like the adrenals of normal rats so treated, they showed depletion of the lipid in the zona glomerulosa (fig. 6).

In view of the reports by Ludden, Krueger and Wright<sup>42</sup> and Durlacher, Darrow and Winternitz<sup>21</sup> concerning the hypertrophy of the kidneys following administration of desoxycorticosterone acetate, the renal weights of the 6 animals in this group were determined. Koren-

40. Hashimoto, E. I.: *Anat. Rec.* **81**:205, 1941.

41. Andersen, D. H., and Kennedy, H. S.: *J. Physiol.* **79**:1, 1933.

42. Ludden, J. B.; Krueger, E., and Wright, I. S.: *Endocrinology* **28**:619, 1941.

chevsky and Ross<sup>43</sup> found that in male rats castration resulted in a decrease in the size of the kidneys while in female rats ovariectomy caused no such change. The latter observation is confirmed by the data in table 7. It will be noted that desoxycorticosterone acetate administered to ovariectomized rats produced adrenal hypertrophy similar to that reported by Durlacher and associates.<sup>21</sup>

#### STILBESTROL

The adrenals of 4 normal and 4 hypophysectomized male rats (Yale strain) were examined after the rats had undergone a fifty-four hour fast. Two animals in each group had been given a single injection of stilbestrol (5 mg. per hundred grams of body weight) after the first twenty-four hours of fasting. The weights of the glands are presented in table 8.

TABLE 8.—*Effect of Administration of Stilbestrol on the Weight of the Adrenals of Fasting Normal and Hypophysectomized Males*

Group	Rats	Loss of Body Weight	Adrenal Weight, Gm. per Kg.	
			With Initial Body Weight	With Final Body Weight
Normal.....	2	13%	0.1539	0.1867
Stilbestrol-treated*.....	2	15%	0.1415	0.1739
Hypophysectomized †.....	2	18%	0.1083	0.1338
Hypophysectomized and given stilbestrol†.....	2	18%	0.1045	0.1312

\* The stilbestrol was injected after twenty-four hours of fasting (5 mg. per hundred grams of body weight).

† These animals were put to death fifty-four hours after hypophysectomy.

Relative hypertrophy of the adrenals was observed in both the starved normal and the stilbestrol-treated animals. The single injection of stilbestrol resulted in no absolute enlargement of the adrenals. James and Nelson<sup>38</sup> and Ingle<sup>44</sup> have reported adrenal hypertrophy following repeated injections of stilbestrol. Loeser<sup>39</sup> observed enlargement of the glands and loss of lipid following daily administration of 1 mg. of stilbestrol for two weeks. The glands of the stilbestrol-treated animals in the present small series manifested a moderate degree of depletion of lipid of the zona fasciculata (the sudan appearing as fine droplets) in contrast with the untreated similarly starved controls (figs. 13 and 14). Numerous vacuolated non-lipoid-containing cells were seen in the outer portion of the zona fasciculata. Fry,<sup>45</sup> studying the metabolism of animals given injections of stilbestrol, observed evidence of increased

43. Korenevsky, V., and Ross, M. A.: Brit. M. J. 1:645, 1941.

44. Ingle, D. J.: Endocrinology 29:838, 1941.

45. Fry, E. G.: To be published.

glyconeogenesis, as did James and Nelson<sup>38</sup> in experiments conducted over longer periods.

Hypophysectomy followed by a fifty-four hour fast resulted in considerable atrophy of the adrenal. No marked difference in reduction of lipid content could be discerned between the adrenals of hypophysectomized, stilbestrol-treated rats and those of animals simply hypophysectomized (figs. 15 and 16). Fry found that whereas a single injection of stilbestrol into normal starved animals stimulated glyconeogenesis, there was no evidence of such stimulation in hypophysectomized starved animals. On this basis Fry suggested that the stilbestrol action on the adrenal is mediated through the pituitary. This idea is supported by the work of Bourne and Zuckerman,<sup>12</sup> who showed that the expected adrenal hypertrophy following injection of estrone (theelin) did not occur in hypophysectomized rats. They stated that "the effects of estrogenic stimulation on the adrenals are mediated through the anterior lobe of the pituitary." It is interesting to note the reduction of cortical lipid following a single injection of stilbestrol in only those animals manifesting increased glyconeogenesis.

#### SUMMARY

An attempt has been made to correlate morphologic changes in the adrenal cortex of the rat with various experimentally produced metabolic and endocrine disturbances.

The administration of desoxycorticosterone acetate (2 mg. daily for thirty days) resulted in considerable atrophy of the adrenal in the male but no significant atrophy in the female adrenal. In both, the zona glomerulosa was shrunken and depleted of lipid. Similar depression of the muscle and serum potassium by dietary measures resulted in no significant gross or microscopic changes in the adrenal cortex.

The adrenal atrophy observed following hypophysectomy was seen to be due to shrinkage and disappearance in part of the cells of the zona fasciculata, the zona glomerulosa becoming slightly hyperplastic—i. e., a histologic picture completely different from that seen following the administration of desoxycorticosterone acetate. The latter when given to hypophysectomized animals caused further atrophy of the adrenal, associated with shrinkage and depletion of the lipid of the zona glomerulosa. These observations would indicate that adrenal atrophy following administration of desoxycorticosterone acetate is not mediated through the pituitary gland.

Castration of adult female rats caused no significant atrophy of the adrenal. Administration of desoxycorticosterone acetate to castrated females produced no further alteration in size but produced depletion of the lipid of the zona glomerulosa.



Acute inanition resulted in moderate adrenal hypertrophy in the female and only slight hypertrophy in the male. Depletion of cortical lipoid was seen in about half the animals starved for one week. Hypophysectomized rats suffering from decreased appetite with resulting extreme loss of weight over a four week period exhibited adrenals smaller than those of comparatively well nourished hypophysectomized animals. This would indicate that factors other than lack of adrenotropic hormone may be responsible for the adrenal atrophy seen in chronic inanition.

The adrenals of rats fed a diet rich in protein were markedly enlarged and showed some degree of lipoid depletion. The latter was characteristic of the adrenals of rats receiving a single injection of stilbestrol. It is suggested that adrenal hypertrophy and lipoid depletion are indicative of the increased protein catabolism observed in acute inanition, with increased consumption of protein and after administration of stilbestrol.

# PARVILOCULAR TUMORS OF THE OVARY

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The majority of the investigations of ovarian tumors which have been published during the last twenty years have been devoted to the so-called rare solid tumors—the granulosa cell tumor, the dysgerminoma and the arrhenoblastoma. The cystic tumors, which statistically form the majority of all ovarian neoplasms, have been left in the background. However, these tumors which by frequency and size aroused much interest among gynecologists and pathologists during the last two decades of the nineteenth century still offer many interesting and unsolved problems. Some special types of cystic tumors have been entirely neglected in the recent literature. Among these is a type of cystoma the classification of which as a specific entity goes back to Pfannenstiel,<sup>1</sup> who in 1907 described a special type of ovarian adenoma which he called a “solid” adenoma.

This “solid” adenoma, according to his definition, is an ovarian tumor which grossly looks solid but microscopically presents innumerable small adenomatous or tubular formations embedded in a fibromatous stroma. This type of structure can be found in several groups of ovarian tumors. Pfannenstiel was the first to call this tumor parvilocular cystoma. The first case of his new group he described as follows:

. . . . In pseudomucinous cystomas of larger size sometimes solid parts can be found, which microscopically consist of very small cystic cavities lined by one row of typical pseudomucinous epithelium and separated from each other by a small amount of connective tissue. The little cysts contain pure pseudomucin. Sometimes the “solid” part predominates over the cystic, so that the tumor correctly may be called a “solid” adenoma.

Pfannenstiel mentioned a patient with a tumor of this type operated on by Werth: A 38 year old quadripara presented on the right side a pedunculated ovarian tumor weighing 10 Kg. It had a smooth surface and consisted partially of small cysts and partially of “solid” portions. The “solid” parts, which he illustrated beautifully in color, presented the typical parvilocular structure: small cystic cavities lined with pseudomucinous columnar epithelium. The tumor was histologically

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1. Pfannenstiel, J., in Veit, J: *Handbuch der Gynäkologie*, ed. 2, Wiesbaden, J. F. Bergmann, 1907, vol. 1, pt. 4.

and clinically noncancerous, and the patient was found to be in good condition fifteen months after the operation. Pfannenstiel mentioned 2 cases of this type in his own material. In the first the bilateral tumors had the size of a fist and there was abundant ascites; in the second case the left ovary had the size of a hen's egg, whereas the right was represented by a pseudomucinous cystoma the size of a man's head. Both patients were in perfect health ten years after operation.

Thus far, the parvilocular cystoma—a term which seems to be better than “microcystic cystoma” because it avoids repetition—seems to be well defined and characterized by the formation of a grossly solid parenchyma consisting of innumerable microscopic cystic cavities lined by a single row of mucin-producing epithelium and embedded in a scarce fibrous stroma. Pfannenstiel cited only 1 other case from the literature, the case reported in 1905 by Glockner.<sup>2</sup> That case, the classification of which has been viewed with much doubt by later investigators, will be analyzed later. Kermauner<sup>3</sup> in 1932 discussed the parvilocular cystoma. I have been unable to find any case reports or discussions of the parvilocular cystoma in the interval between Pfannenstiel's and Kermauner's presentations. Kermauner cited a case published in 1870 by Waldeyer,<sup>4</sup> but in that case carcinoma of the stomach developed simultaneously. The ovarian tumor, according to the description and the illustration, probably was a deposit of this carcinoma and not primary. Today pathologists would call it a Krukenberg tumor.

Kermauner pointed out that at the second gynecologic clinic of the University of Vienna in thirty years no patient with parvilocular cystoma had been operated on. To fill this gap he described, offering four illustrations, a case which he called the Woyer case.<sup>4a</sup> After operation the specimen was submitted to me. The tumor had developed several years after the menopause and caused signs and symptoms of a mechanical type, being hard and resistant like wood and reaching the size of a man's head. The surface was smooth and slightly bosselated. On transverse cut the neoplasm showed a few little cystic cavities in a firm, solid fibroma-like stroma. Through a magnifying lens, innumerable pinpoint-sized cavities became visible, resembling closely the structure of dense pumice stone. Microscopic sections revealed a typical microcystic structure. The little round and oval cavities were lined with one row of epithelial cells varying in type from low columnar to cuboidal (fig. 1). The upper part of the protoplasm of this epithelium

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2. Glockner, A.: *Arch. f. Gynäk.* **75**:49, 1905.

3. Kermauner, F., in Veit, J., and Stoeckel, W.: *Handbuch der Gynäkologie*, ed. 3, Munich, J. F. Bergmann, 1932, vol. 7, pt. 3.

4. Waldeyer, W.: *Arch. f. Gynäk.* **1**:252, 1870.

4a. He meant that the patient had been operated on by Dr. Woyer.

and the contents in the lumens of the cavities gave positive reactions when stained with mucicarmine. The patient, as far as I know, was in good health eight years after the operation.

In his résumé Kermauner said that thus far no case of cancerous parvilocular cystoma had been observed. The next and until today the last discussion of the parvilocular cystoma was given by Miller,<sup>5</sup> in 1937. In his classification of ovarian tumors he described the cases enumerated by his predecessors and some additional cases which probably belonged to other groups. Frankl's case 16, called by Frankl<sup>6</sup> himself a case of "fibroma ovarii adenocysticum pseudomucinosum" is not one of parvilocular cystoma, and his case 15, called by him a case of "fibroma ovarii adenocysticum carcinoides serosum, partim pseudomucinosum," is a typical case of Brenner tumor. Amann's<sup>7</sup> case

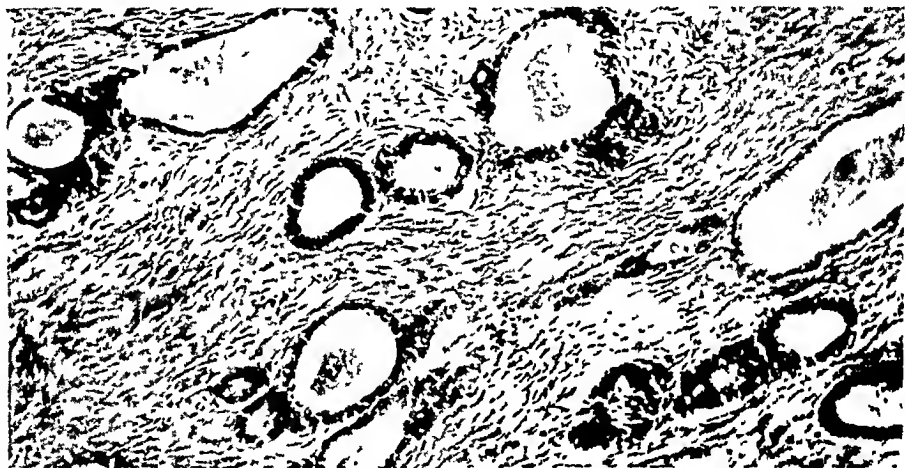


Fig. 1.—Dense fibromatous stroma with small cysts in the Woyer case. Some of the cysts are round; some are stretched by the surrounding fibroma-like bundles. The epithelium is low cuboidal with relatively large nuclei.

showed small cysts lined by mucin-producing high columnar epithelium, but this epithelium is shed and dissolved in its own secretion, which in some places penetrates the stroma and imbibes it. These two changes are typical for the pseudomyxoma or for Krukenberg's tumor but are not described in the parvilocular cystoma. Only the case reported by Orthmann,<sup>8</sup> as far as gross appearance of the tumor is concerned, may belong

5. Miller, J., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1937, vol. 7, pt. 3.

6. Frankl, O.: *Arch. f. Gynäk.* **131**:325, 1927.

7. Amann, J. A., Jr.: *Kurzgefasstes Lehrbuch der mikroskopisch-gynäkologischen Diagnostik*, Wiesbaden, J. F. Bergmann, 1896; *Monatschr. f. Geburtsh. u. Gynäk.* **5**:224, 1897.

8. Orthmann, E. G.: *Monatschr. f. Geburtsh. u. Gynäk.* **9**:771, 1899.

to this group: a bilateral tumor, grossly solid, microscopically microcystic, pseudomucinous and clinically noncancerous. But here, too, the columnar epithelium dissolves in its secretion and many of the cystic cavities present no epithelial lining but have a plug of mucinous secretion embedded in the fibrillar stroma.

For the reason that probably different types of tumors are placed under the same heading, the statistics of some authors, as Lippert,<sup>9</sup> Kusuda<sup>10</sup> and Stratz,<sup>11</sup> are not reliable as far as the incidence of the parvilocular cystoma is concerned. "Solid" adenoma and "pseudosolid" cystoma evidently are confused, and solid adenoma, as well as the combination types of cystoma and fibroma, or cystofibroma or adenofibroma, admitted. This probably is the explanation why Stratz, for instance, finds that solid adenoma forms 3 per cent of all epithelial neoplasms of the ovary. If only those tumors are accepted as parvilocular cystoma which present the characteristics given by Pfannenstiël, this type of tumor proves to be rather rare, as has already been pointed out by Kermauner. In the cases reported by Pfannenstiël, Amann and others the follow-up observations to prove that the neoplasms were clinically noncancerous were carried on for only a few years. The illustrations are more or less diagrammatic, simplified drawings, most of which give no accurate picture of the cellular structure. Only the presentation of Kermauner is illustrated by three modern photomicrographs and a colored painting of the gross tumor; the follow-up is limited to six years. and neither the history nor the autopsy report are given.

#### REPORT OF CASES

CASE 1.—Dr. C. E. Galloway, Evanston, Ill., supplied the history and Dr. E. L. Benjamin, Evanston, Ill., the slides of this case. Mrs. H., 38 years old a Danish-born white woman, was admitted because of pain in the rectum. A left ovarian tumor was diagnosed and removed in September 1937, in the Evanston Hospital. Pain, with some loss of weight and fatigue, returned, and in December 1937 the remaining ovary and the uterus were removed. She did poorly, and pain developed along the right costal margin. On admission she was pale and thin. The heart and lungs were normal; the urine was normal; the Wassermann and Kahn tests were negative; the hemoglobin was about 60 per cent; the red blood cells numbered 3,400,000 and the white cells 8,000 per cubic millimeter. Cystoscopic and roentgen examinations gave negative results. The temperature was normal. She received 9,000 roentgens (r) to six abdominal portals and one transfusion, with some improvement, and was discharged in February 1938. In April she was readmitted, considerably emaciated, with marked abdominal tenderness and rigidity. The red blood cell count was 3,000,000; the white cell count, unchanged.

9. Lippert, W.: *Arch. f. Gynäk.* **74**:389, 1904.

10. Kusuda, S.: *Arch. f. Gynäk.* **124**:269, 1925.

11. Stratz, C. H.: *Gynäkologische Anatomie: II. Die Geschwülste der Eierstöcke*, Berlin, H. Kornfeld, 1894.

She received an additional 3,500 r to four upper abdominal portals but declined rapidly, with nausea, loss of weight and weakness, but without fever. She died in June 1938.

*Specimen Obtained at the First Operation* (left ovary and tube, examined by Dr. E. L. Benjamin).—The tube was normal with several serosal cysts at the tube and the mesosalpinx. The ovary measured 11 by 8.5 by 9.5 cm. It was covered by an intact, thickened, smooth, pearly gray to yellow-pink capsule. Numerous dilated and engorged vessels were seen within the capsule. The consistency was firm and the contour slightly bosselated. Cross section revealed a firm to semi-cystic, coarsely lobulated, yellowish green to pink-yellow, slippery, sticky tumor enclosed by a capsule and attached over an area of 8 by 9 cm. The internal lining

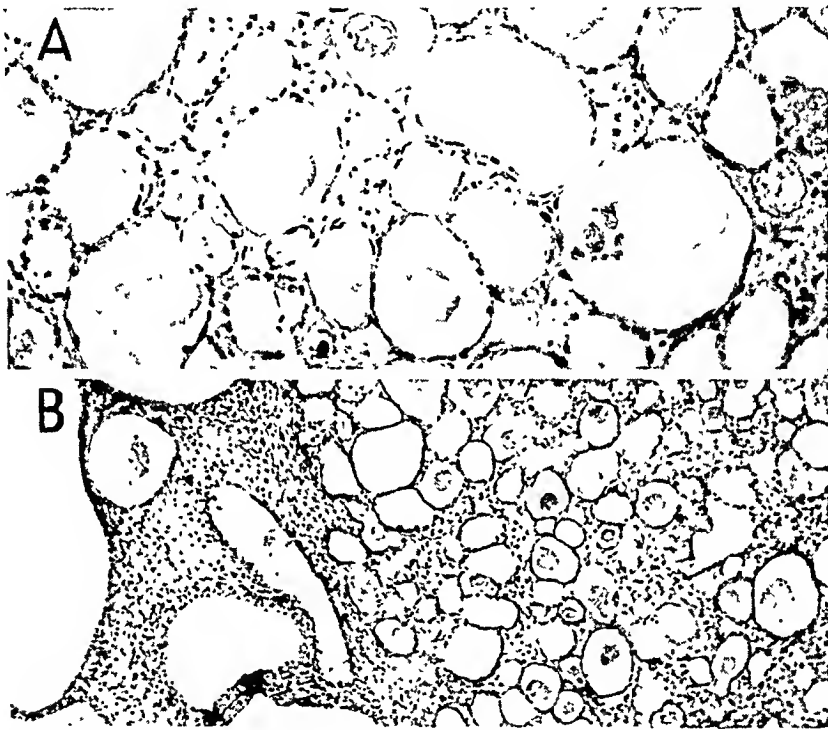


Fig. 2 (case 1).—Section of an ovarian tumor with small cysts as the outstanding structural feature; the stroma is poorly developed. The cysts are lined with low cuboidal or flattened epithelium. *A*, medium enlargement; *B*, low enlargement.

of the capsule varied from tissue paper thinness to a thickness of 2 mm., and it was incompletely studded by elevated translucent, glistening, white, discrete and confluent cysts.

The cut surface of the main tumor showed a firm, slippery and slightly sticky solid tumor through which passed yellow-gray streaks of fibrous tissue; other parts of the tumor were honeycombed and composed of numerous cysts filled with a sticky fluid. The walls were practically colorless. Still other solid areas of the tumor presented irregularly firm, pale, greenish yellow to pink-yellow areas. There was a moderate number of blood vessels coursing over the tumor, arising from its base and attached to the interior of the capsule.

Microscopically, the tumor presented different structures. Most characteristic were the parts which grossly looked solid. Here, corresponding exactly to the description and the illustration given by Pfannenstiel, were closely packed small round or oval cystic cavities, separated only by thin septums of fibrous stroma and lined by a single row of cuboidal or even flattened epithelial cells with vesicular nuclei and pale-staining protoplasm. These microcystic cavities presented two variations. In some areas they were larger and polyhedral. The single cavities had a diameter up to 8 mm. The larger ones were therefore visible to the naked eye. As the epithelium became higher and definitely cylindric, the upper part of the protoplasm was engorged by a pale secretion. In many cells the top was crowned by a crescent-shaped, markedly projecting drop of secretion. In some of the largest cavities proliferation of the epithelium resulted in a second and even a



Fig. 3 (case 1).—*A*, transition from a cystic area into a solid medullary area; *B*, the solid medullary area. Note the irregularity of the nuclei, which are partly hyperchromatic.

third and a fourth row of cells. In other areas the cavities appeared smaller, the lumens being reduced in size by marked enlargement of the cells, which were of irregular shape and size and possessed pale protoplasm and hyperchromatic nuclei. In this way solid masses of epithelial cells were formed. These epithelial masses showed the carcinomatous character of the tumor in the irregular and atypical character of the individual cells and by invading and replacing the fibrous stroma. In some areas, where larger cavities were present, a third change could be noticed. The epithelial lining by piling up formed papillomatous projections which in the areas of greatest active proliferation filled out the cavities. These papillomatous projections also presented the cellular irregularity of a carcinomatous growth. As

a further proof of carcinomatous invasion, the same projections were found on the serosa of the uterus, on the perimetrium and on the surface of the second ovary. Here little clusters of dark-staining epithelial cells were embedded in masses of fibrin that covered the surface of the ovary. These represented the process of metastatic grafting to the surface, whereas in other areas there were papillomatous or small cystic deposits in the albuginea. In the small cysts the secretion in the lumen and in the upper parts of the cells gave a mucin reaction when stained with

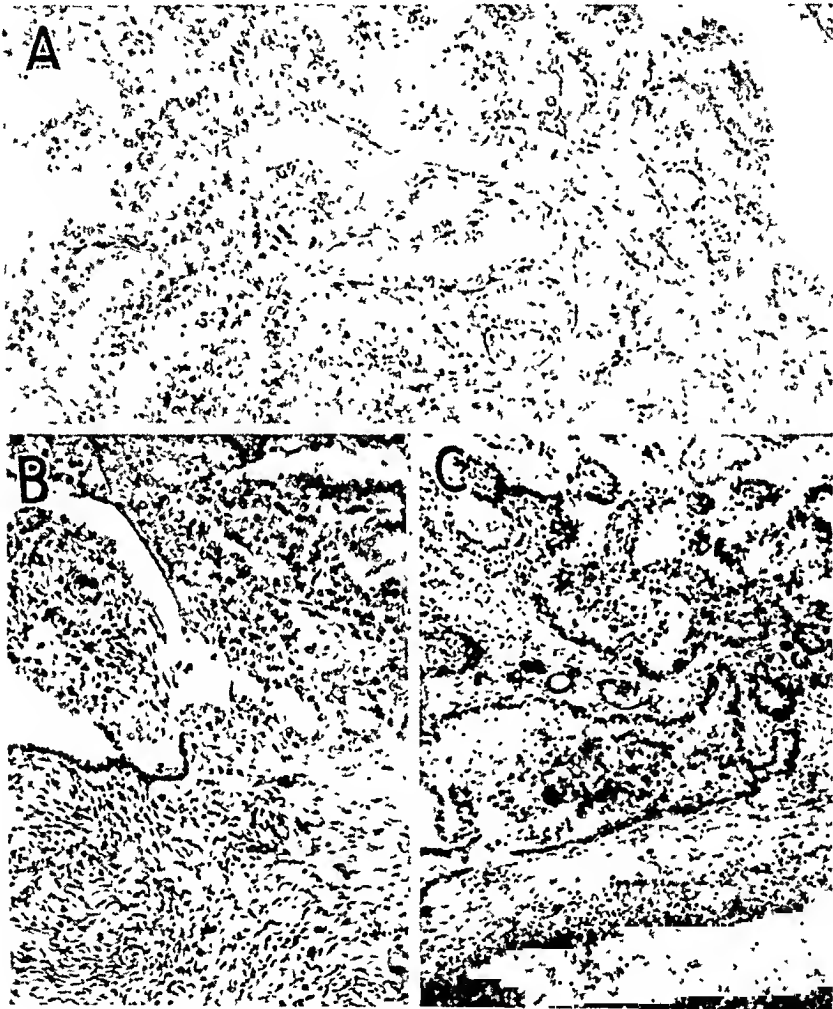


Fig. 4 (case 1).—*A*, area with adenopapillary structure; *B*, implantation of papillary deposits on the surface of the second ovary; *C*, implantation of papillary tumor masses on the thickened capsule of the spleen.

mucicarmine. Wherever the epithelial lining formed solid masses or papillary projections, the production of mucin gradually diminished and finally stopped (figs. 2, 3 and 4*A* and *B*). Clinically, the patient gave full evidence of the carcinomatous character of this neoplasm, which was suspected after the first operation and became manifest in the deposits encountered at the second operation.

*Autopsy* (Dr. A. Brunschwig and Dr. N. B. Friedman, Albert Merritt Billings Hospital, Chicago).—All the abdominal viscera were matted and rendered adherent



to one another and to the parietes by fibrous and fibrinous adhesions and located between them were collections of fluid varying in color from greenish to dark brownish turbid and amounting to about 1,000 cc. The entire free peritoneal cavity was lost, the visceral and parietal surfaces being approximated and the cavities filled in by cystic tumor tissue and confluent masses bound together by dense fibrous and fibrinous adhesions. The intestinal loops were matted together, as were the pelvic viscera. The subdiaphragmatic spaces were similarly obliterated. The liver, gallbladder, spleen and pancreas were embedded in tumor tissue.

The abdominal as well as the periesophageal lymph nodes presented complete replacement of their parenchyma by tumor tissue. Microscopically, the tumor tissue consisted of proliferating papillary masses which morphologically duplicated the tumor tissue found at the perimetrium at the second operation (fig. 4C).

In its characteristic pseudosolid parts the tumor just described duplicates in every respect the description and definition given by Pfannenstiël. Small cystic cavities lined with mucin-producing low epithelium formed the specific structural unit. The change to larger cavities and papillomatous proliferation brings the tumor in relation to the common pseudomucinous cystoma, which forms larger, grossly visible cavities and eventually becomes papillomatous. This type ordinarily has higher and more regular columnar epithelium, which morphologically is identical with cervical epithelium. It may be that there is a relation between the height of the epithelium and the size of the cystic cavities such that high epithelium has both the tendency and the faculty to form large cavities, whereas low cuboidal epithelium tends to form small cavities. Whereas all the tumors of this type described by Pfannenstiël and Kermauner were noncancerous, this one pathologically, microscopically and clinically gave definite evidence of carcinomatous change. This is in accord with the general experience and conception that all types of ovarian tumors are primarily noncancerous but may change secondarily into cancer.

CASE 2.—From Dr. Milton Bohrod I received six paraffin blocks of an ovarian tumor. Unfortunately, the history was lost, but in spite of this the histologic picture deserves comment (fig. 5). It represents a parvilocular cystoma with changes into papillary proliferation and into carcinoma as in the previous case. The only difference is that in some areas the single cells are markedly flattened, on both ends, so that the nucleus in the middle projects toward the lumen. The cells thus become hobnail shaped, the epithelium endothelium-like and the whole structure similar to that of a mesonephroma. However, the specific mesonephromatous glomerulus-like units are missing, and the mucin reaction is strongly positive, whereas that in the typical mesonephroma is negative.

CASE 3.—Dr. L. Loeffler, pathologist of the Decatur and Macon County Hospital, Decatur, Ill., gave me the data on this case. A 69 year old white woman took sick four to five days before she died, with signs of intestinal obstruction.

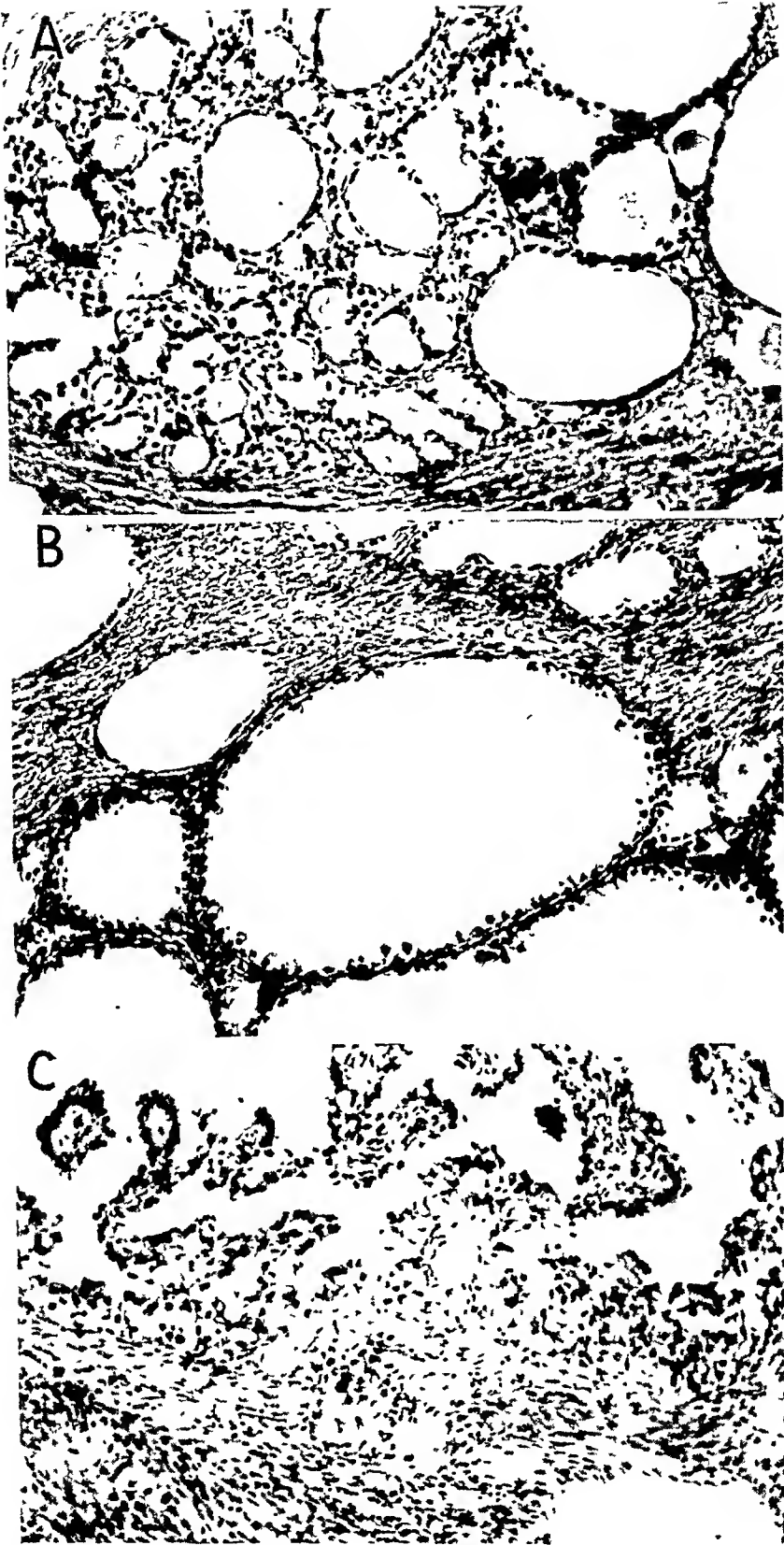


Fig. 5.—Sections of tumor in case 2: *A*, small cystic area; *B*, larger cysts, the stretched epithelium of which duplicates the hobnail or mushroom-shaped cells of mesonephroma; *C*, papillary area.

Roentgen examination showed a large soft tissue mass in the left side of the abdomen and the sigmoid narrowed where it crossed this mass. Removal of the tumor was not considered because of the bad condition of the patient. Two days after admission a small incision was made and a cystic part of the tumor emptied.

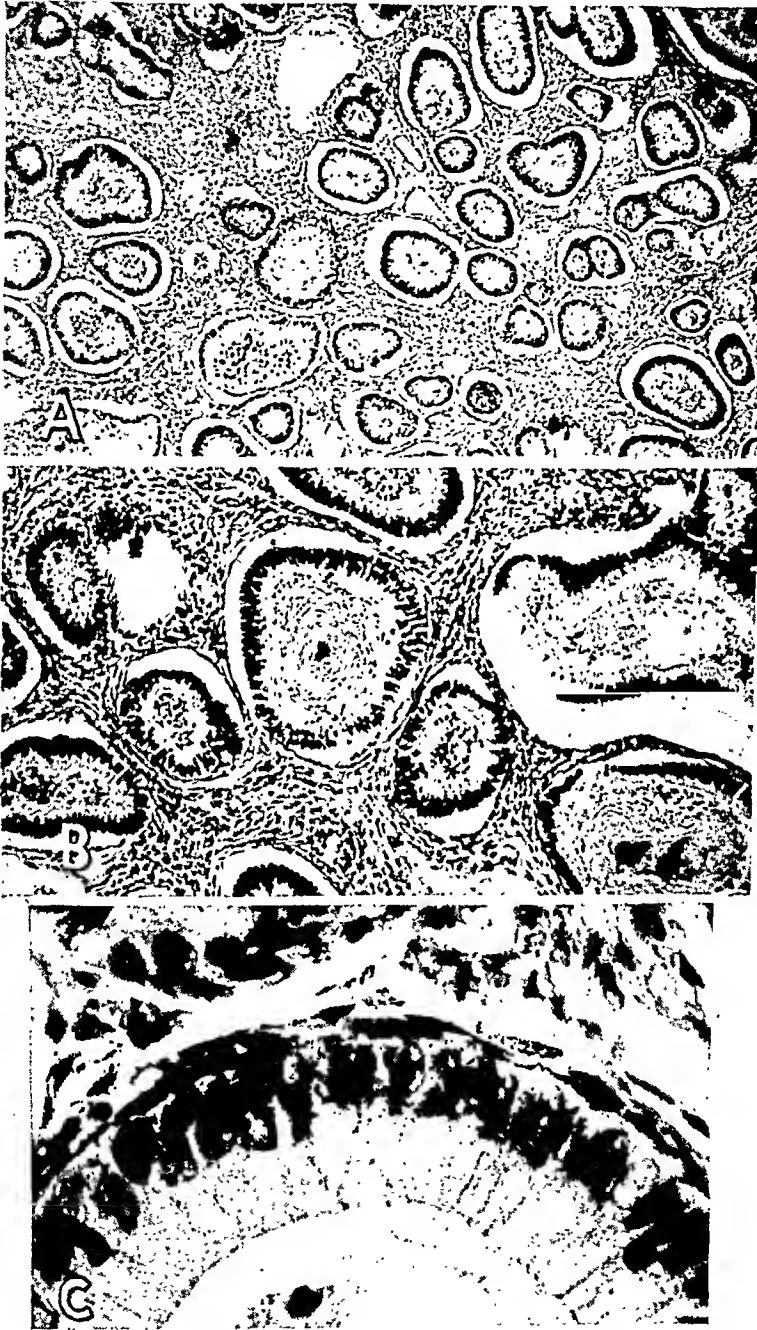


Fig. 6 (case 2).—Small round cysts lined with high columnar epithelium and embedded in well developed fibromatous stroma. (The separation of the epithelium from the stroma indicates that necrosis is beginning in consequence of the twisting of the pedicle.) *A* shows low enlargement, *B* medium enlargement and *C* high enlargement.

The patient died with symptoms of intestinal obstruction, heart failure and what was believed to be a twisted ovarian cystoma. The autopsy revealed a freely movable pelvic tumor about the size of a man's head, connected with veil-like fibrinous membranes to the surrounding tissues. The tumor was infarcted with mostly clotted dark red blood in its several chambers. The surface of the tumor was smooth, bluish and bloody and covered by a fibrous membrane. On the cut surface numerous smaller cysts were seen, about 4 by 5 cm. in diameter, all filled with blood. The solid masses were relatively soft and cut easily, and the cut surfaces were finely granular. The color of these masses was grayish pink. They were sharply defined within the limit of smaller cysts, about 3 by 4 and 2 by 2 cm. in size.

The tumor represented the right ovary and was twisted around its pedicle counterclockwise from the right over to the left abdominal side above the pelvis. The pedicle was made up of the broad ligament and the tube, which were deep red and hemorrhagic. The uterus was small and showed several small subserous fibroids about 1 cm. in diameter. The intestines were all pushed over to the right side of the abdomen, especially the sigmoid and the small intestines. The sigmoid crossed the abdominal cavity from the upper left to the lower right quadrant.

Microscopically, the tumor showed solid parts, which duplicated a moderately cellular fibroma, and cystic parts, which were composed of irregularly shaped cavities. The largest cavities were about 5 cm. in diameter. From these large cavities there were gradual transitions to the smallest, which measured 0.01 mm. in diameter. In large areas of the tumor the tissue was studded with small cysts, thus presenting the typical picture of the grossly solid, microscopically parvilocular cystoma (fig. 6). The lining of the cavities consisted of high columnar epithelium with oval nuclei filling the basal part of the cells. The protoplasm of the columnar cells, as well as the contents of the cavities, gave a strong mucin reaction. As a consequence of the twisting, extensive areas of the tumor were necrotic and congested with red blood corpuscles, and even in the well preserved areas the damage to the tissue was indicated by the separation of the epithelium from the stroma. There was no indication of carcinomatous change.

The tumor just described was a fibrocystoma with relatively small compartments, which by growing still smaller and being reduced to a size far beyond gross visibility formed the typical structure of a parvilocular cystoma. The fibrous stroma together with the large and small cavities forms the main part of the tumor tissue. These cavities are separated from one another by strong broad fibrous walls and not by paper-thin septums such as those seen in the multilocular serous or pseudomucinous cystoma. Occasionally a small area presenting the mucinous type of epithelium resembles closely the pseudomucinous cystoma. But in tumors of the latter type the columnar epithelium is higher and more regular and the protoplasm stains paler than in this case.

#### DIFFERENTIAL DIAGNOSIS

Pfannenstiel mentioned that pseudosolid structure can be found in ovarian tumors of different types, and Kermauner<sup>3</sup> in his book has an illustration (no. 88) of a pseudomucinous cystoma with a pseudosolid

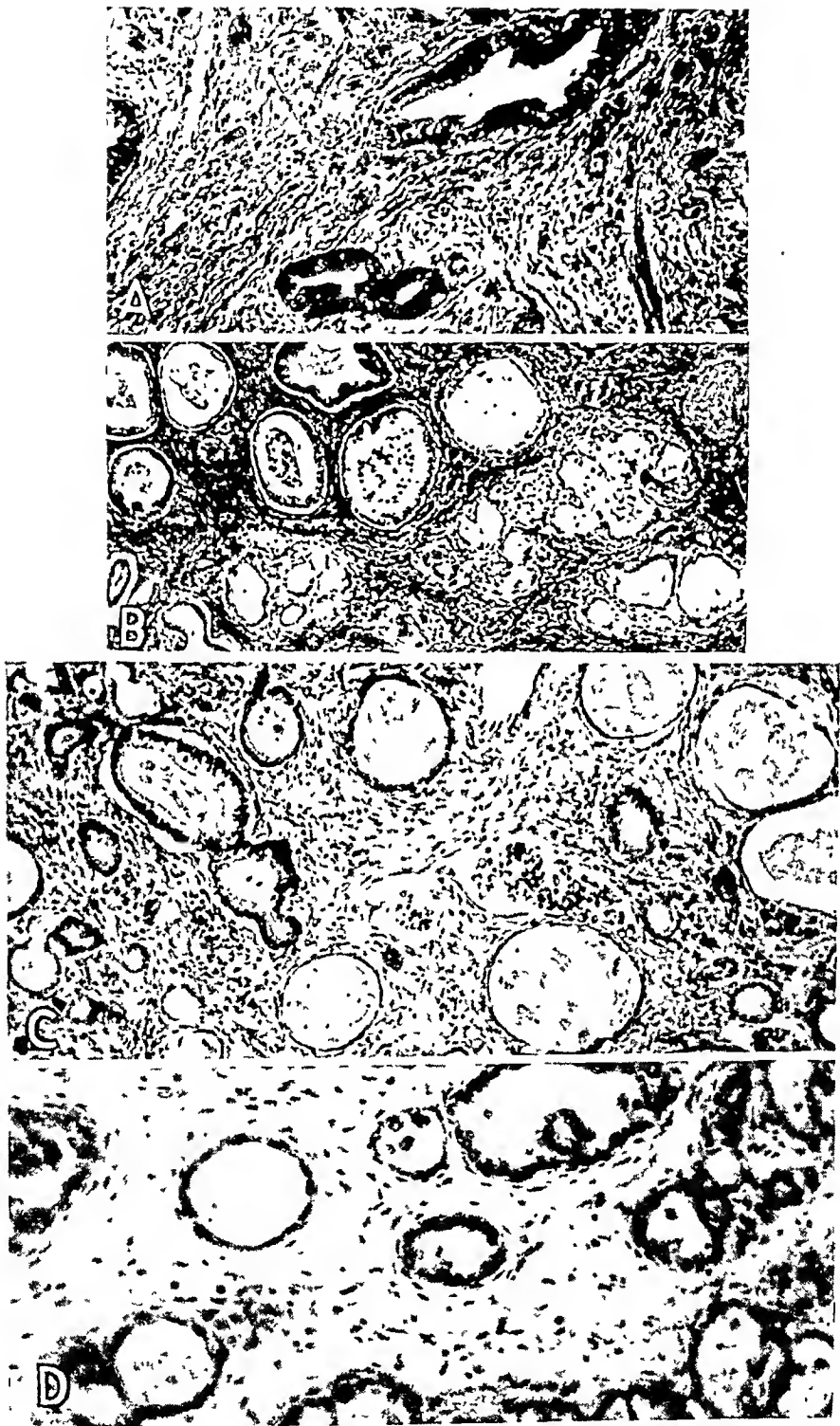


Figure 7

(See legend on opposite page)

node in the center, which is not carcinomatous. Unfortunately, there is no histologic description in his text. Miller<sup>5</sup> described similar findings and cited cases described by Glockner,<sup>2</sup> Frankl<sup>6</sup> and Meyer.<sup>12</sup> A characteristic example of this type is in my collection:

A 61 year old woman showed the right side of the abdomen dilated by a smooth cystic ovarian tumor the size of a man's head, with solid immobile firm nodes in the pouch of Douglas. By laparotomy complete hysterectomy was performed, but the peritoneum of the vesicouterine fold and of the pouch of Douglas was found infiltrated and indurated by tumor tissue. On examination of the specimen the uterus was found to be of normal size and shape with polypoid hyperplasia of the endometrium, the tubes normal and the left ovary transformed into a multilocular pseudomucinous cystoma with a smooth surface. The right ovary was the size of a plum and showed on the transverse cut surface numerous, irregularly shaped small cystic cavities filled with mucin. On section the large tumor showed in its central basal part a fibrous thickening of the intercystic septums which had fused with remnants of ovarian tissue. The peripheral portion showed large cystic cavities lined with pseudomucinous, rather polymorphous columnar epithelium. This epithelium by its irregular and atypical elements gave evidence of carcinomatous change. In the basal area solid fibroma-like masses of stroma were found, which were studded with round cystic and elongated glandlike cavities lined with the same pseudomucinous carcinomatous epithelium. These cavities were seen in the periphery of some of the corpora candicantia and adjacent to the perivascular lymph spaces of some medium-sized arteries, as a result of carcinomatous invasion. The tumor in its central part grossly duplicated the parvilocular cystoma. Its histogenesis, however, was definitely different. It represented a combination tumor: a multilocular pseudomucinous cystoma plus a fibroma, with secondary carcinomatous change of the pseudomucinous epithelium, which finally invaded the fibromatous part of the tumor (fig. 7 *A* and *B*).

This tumor definitely belongs to a group of ovarian neoplasms different from the parvilocular cystoma. It is similar to the latter only in the gross appearance of some of its parts. Histologically and histogenetically it represents a different entity and has to be classified as pseudomucinous papillary fibroadenocystoma with secondary carcinom-

12. Meyer, R.: *Monatschr. f. Geburtsh. u. Gynäk.* **44**:302, 1916.

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#### EXPLANATION OF FIGURE 7

*A* and *B*, carcinomatous fibrocystoma: *A* shows carcinomatous tubules close to the hilus and *B* small cysts at the hilus lined with columnar or cuboidal mucinous epithelium and embedded in fibrous stroma.

*C*, Krukenberg's tumor: The section is from a deposit of carcinoma in the ovary following metastasis of a carcinoma of the stomach to this site. Small cysts characterize it; the lining epithelium shows transitions from high columnar to flat, linear epithelium. A lymph space to the right from the center contains floating signet ring cells. The stroma is invaded by single carcinoma cells and small clusters.

*D*, carcinomatous mesonephroma of the ovary with single and confluent small cysts.

atous changes. Pseudosolid structures can be found in many other types of ovarian neoplasms which have to be separated from the parvilocular cystoma.

*Multilocular Pseudomucinous Cystoma.*—Here the cavities are much larger and may reach a diameter of several centimeters. But sometimes they may remain small and may form pseudosolid nodes of a spongy structure. Microscopically, the single cavities on these nodes are polyhedral, forming a honeycomb-like network, and the interstitial tissue is limited to a few connective tissue fibers in the thin septums between the cavities—the true parvilocular cystoma has a well developed fibroma-like stroma in which the round cavities are embedded. The epithelial lining of the pseudomucinous cystoma is high columnar; the epithelium of the parvilocular cystoma is much lower and may even be flat. The epithelium of the pseudomucinous cystoma eventually secretes distinct drops in goblet cells; in the parvilocular cystoma the mucinous secretion diffusely fills the upper part of the protoplasm.

When becoming papillomatous, the pseudomucinous cystoma in general forms large projections with a well developed stock of massive fibrous tissue. The parvilocular cystoma forms fine or thin ramifying projections which consist mainly of epithelium arranged around a thin core of a few connective tissue cells.

*Krukenberg's Tumor.*—This tumor sometimes presents areas composed of round little cysts scattered over the proliferated dense fibromatous stroma. Here the presence of primary carcinoma of the stomach, of the intestines or the gallbladder is of decided importance for the differential diagnosis. Histologically, the Krukenberg tumor shows diffuse infiltration of the stroma by carcinoma cells, which by secretion may develop into signet ring cells; it shows carcinoma cells floating in lymph spaces and eventually signet ring cells in the lining of the cavities, which may be shed and dissolved in the mucin secreted in the lumen; such changes are not found in the parvilocular cystoma (fig. 7 C and D).

*Mesonephroma.*—The mesonephroma may form a network of small cavities lined with low flat endothelial-shaped cells. The similarity of the mesonephroma to the parvilocular cystoma is great, and it is likely that some ovarian tumors diagnosed and described as mesonephroma are true parvilocular cystomas. However, such a misclassification can be avoided by diagnosing as mesonephroma only tumors which present the specific structures of this entity, none of which can be found in the parvilocular cystoma: (1) the glomerulus-like unit, consisting of a small cystic cavity which contains one capillary loop, which is covered with columnar epithelial cells whereas the cavity is lined with low endothelium-like cells; (2) solid areas consisting of proliferated endothelial cells, which appear stellate and are connected with each other by fine filiform

projections. These two specific structures are definitely free from mucin, whereas the parvilocular cystoma shows distinct and even marked production of mucin. However, limited production of a mucinous secretion can be found in the small cystic cavities of the mesonephroma<sup>13</sup> in some cases, but the true mesonephroma never shows a mucinous secretion comparable to that of the pseudomucinous cystoma and the parvilocular cystoma. Staining with mucicarmine or thionine should never be omitted for differential diagnosis of mesonephroma. In the mesonephroma the stroma never develops sufficiently to form fibroma-like areas such as those found in the parvilocular cystoma. Although the linings of the small cavities in both tumors may sometimes have a confusing similarity, the true epithelium, with distinct contact lines between neighboring cells, of the parvilocular cystoma (even if the protoplasm is lower than the projecting nucleus) can be distinguished from the endothelial cells of the mesonephroma, which touch each other only by their sharpened ends (fig. 7 C and D).

*Fibrocystoma.*—The fibrocystoma which is discussed by Pfannenstiel and by Miller in connection with the parvilocular cystoma contains no pseudosolid parts, but solid parts only, which represent the fibroma component of this mixed ovarian tumor. Here one finds not only thickening of the septums between the cystic cavities but well developed masses of solid connective tissue identical with the tissue of which the pure fibroma consists. The epithelial lining of the cavities is identical with the lining of the serous cystoma and consists of columnar cells with cilia or else of nonciliated columnar cells which secrete a serous fluid. This epithelium duplicates the tubal mucosa. Frequently the fibrocystoma produces papillomatous projections in the walls of the cysts and at the outer surface. These projections are plump and massive and have a heavy core of fibrous stroma, in contrast with the fine or thin ramifying papilli with scarce stroma found in the parvilocular cystoma. The fibrocystoma sometimes shows glandlike folds of the epithelium which extend into the stroma. These are never found in the parvilocular cystoma. The accurate name for the fibrocystoma for classification is "serous fibrocystoma," eventually with the attribute "papillary," "adenomatous" or "adenopapillary" (fig. 8).

*Other Types of Ovarian Tumors for Differential Consideration.*—For the histogenesis of the parvilocular cystoma experience and observations are still missing. The faculty of its epithelium to produce mucin brings it in close relation to the pseudomucinous cystoma, which is supposed to develop from inclusions of the ovarian surface epithelium with potency for prosoplastic changes in the direction of müllerian cervical mucosa. However, the production of mucin is not specific and

13. Schiller, W.: Am. J. Cancer 35:1, 1939.



has been observed also in the epithelium of the rete ovarii. The possibility of papillomatous proliferation is one more property the pseudomucinous and the parvilocular cystoma have in common. For the time being it may be justified, further investigation pending, to place parvilocular cystoma in the classification of ovarian tumors as a subgroup of pseudomucinous cystoma.



Fig. 8—Fibrocystoma of the ovary: *A*, large cystic cavities in a well developed fibroma-like stroma. The folds when cut across simulate little cysts. Beneath the low cuboidal epithelium a small zone of pseudoxanthomatous cells has developed. *B*, high columnar, ciliated serous epithelium with dense protoplasm and elevated nuclei. *C*, high columnar mucinous epithelium with light protoplasm and short oval nuclei. *D*, serous columnar cells mixed with goblet cells.

As mentioned, Pfannenstiel<sup>1</sup> cited in addition to his observations only 1 other case, the case published by Glockner<sup>2</sup>:

This was a right-sided ovarian tumor, the size of a man's head, removed by laparotomy from a 47 year old tripara with normal adnexa uteri on the left side.

It measured 22 by 14 by 9 cm. and on section was perfectly solid. Sections revealed a fibromatous stroma with densely arranged, partially racemose glandular ducts lined by a low epithelium of probable mucinous character. These ducts presented a fanlike arrangement, being separated by septums which radiated from the capsule.

Whether this case is to be classified as one of parvilocular cystoma seems doubtful. Kernauner<sup>3</sup> did not accept this classification and suggested calling it an instance of adenofibroma developing from the rete. There is no question that the glandlike ducts of Glockner's illustration

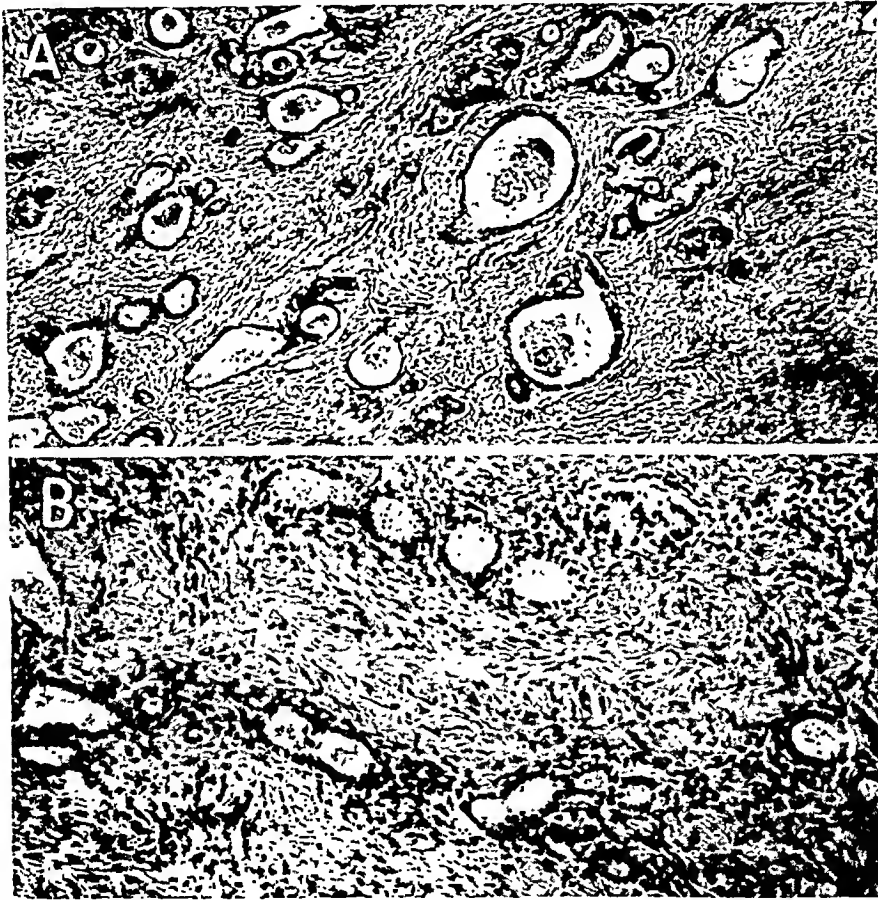


Fig. 9.—Ovarian tumor with fibromatous stroma and small round cysts lined with cuboidal epithelium: *A*, low enlargement; *B*, medium enlargement.

are different from the cystic cavities of the parvilocular cystoma, and, on the other hand, the parvilocular cystoma is not partitioned by radiating septums. Glockner's tumor probably represents a specific entity the characteristics of which are: small racemose-gland-like ducts lined with low columnar or cuboidal epithelium and embedded in a fibroma-like stroma. A specimen similar to Glockner's but without septums is in my histologic collection:

At autopsy on a 77 year old woman who died of lobular pneumonia, complicated by ulcerative esophagitis caused by monilia and atrophy of the right arm following

poliomyelitis, there were discovered incidentally a small serous cystoma of the right ovary and a stony-hard solid tumor, the size of a man's fist, which had replaced the left ovary. This tumor had a smooth surface and grossly gave the impression of a solid fibroma. Microscopically, the fibromatous stroma was densely studded with innumerable narrow, partially racemose ducts lined by cuboidal epithelium. There was little mucinous and considerable serous secretion in the lumens. Fat stains revealed sudanophil droplets in the protoplasm of some of the connective tissue cells in the stroma (fig. 9).

Another case of this group I owe to Dr. E. R. Pund and Dr. Robert Greenblatt, of the University of Georgia:

A Negro woman aged 43 years was admitted to a hospital because of acute heart failure with ascites. A pelvic tumor was found. There had been one child by her first husband and no pregnancies by her second. Her feet swelled on standing. The blood pressure was 200 systolic and 110 diastolic. Paracentesis was done, and 3,500 cc. of greenish fluid was withdrawn and injected into a guinea pig, with a negative result. Following the paracentesis, an indefinite movable mass, the size of a grapefruit, could be felt in the pelvis. Preoperatively there had been no menses for seven years, the menses having suddenly stopped at the age of 37. Three days' spotting was noted before operation. At laparotomy, bilateral firm smooth ovarian tumors, 14 cm. in diameter and well encapsulated, were found. One of these identical tumors contained a compressed simple cyst, 8 by 1 by 3 cm.

The patient was seen one year after operation and was then perfectly well.

Microscopically, the homogeneous fibroma-like stroma contained racemose strands consisting of two rows of epithelial cells, varying in type from cuboidal to columnar. Most of these strands were solid; some, however, formed a small lumen at the blind end. Some of the strands enclosed between the two epithelial rows single large round pale cells, hydropically swollen, with small nuclei like Call-Exner bodies. The strands or ducts were arranged in small groups or lobuli, being separated by more or less distinct septums of parallel fibers of connective tissue which resembled the septums in Glockner's case. Fat stains showed fine sudanophil granules in some of the cylindric cells of the trabeculae and in some of the spindle-shaped cells of the fibromatous stroma. The presence of fat granules in the cells of the strands constituted a similarity to what one finds in some cases of arrhenoblastoma. The fatty degeneration of the stroma cells was entirely different from the lipoid storage in the groups of large polyhedral Leydig cells present in the arrhenoblastoma with endocrine activity.

Here, for differential diagnosis, trabeculated granulosa cell tumor and trabeculated arrhenoblastoma have to be ruled out. In a trabeculated granulosa cell tumor, the cellular elements are round and never columnar, and the trabeculae are composed of more than just two rows of cells and have no tendency to form a lumen. In an arrhenoblastoma, the columnar cells which compose the trabeculae are much higher, the trabeculae are accompanied by Leydig cells, which here are missing, and form only short strands, never a large, well worked-out network as in this tumor. In almost every case of the trabecular granulosa cell tumor or of the trabecular arrhenoblastoma, areas of lower maturity similar to cellular fibroma can be seen with gradual transitions to

trabeculated parts of higher maturity. Such areas were not present in this tumor. Evidently this tumor represented an earlier phase of the glandlike ducts, during which phase they are still solid. This phase

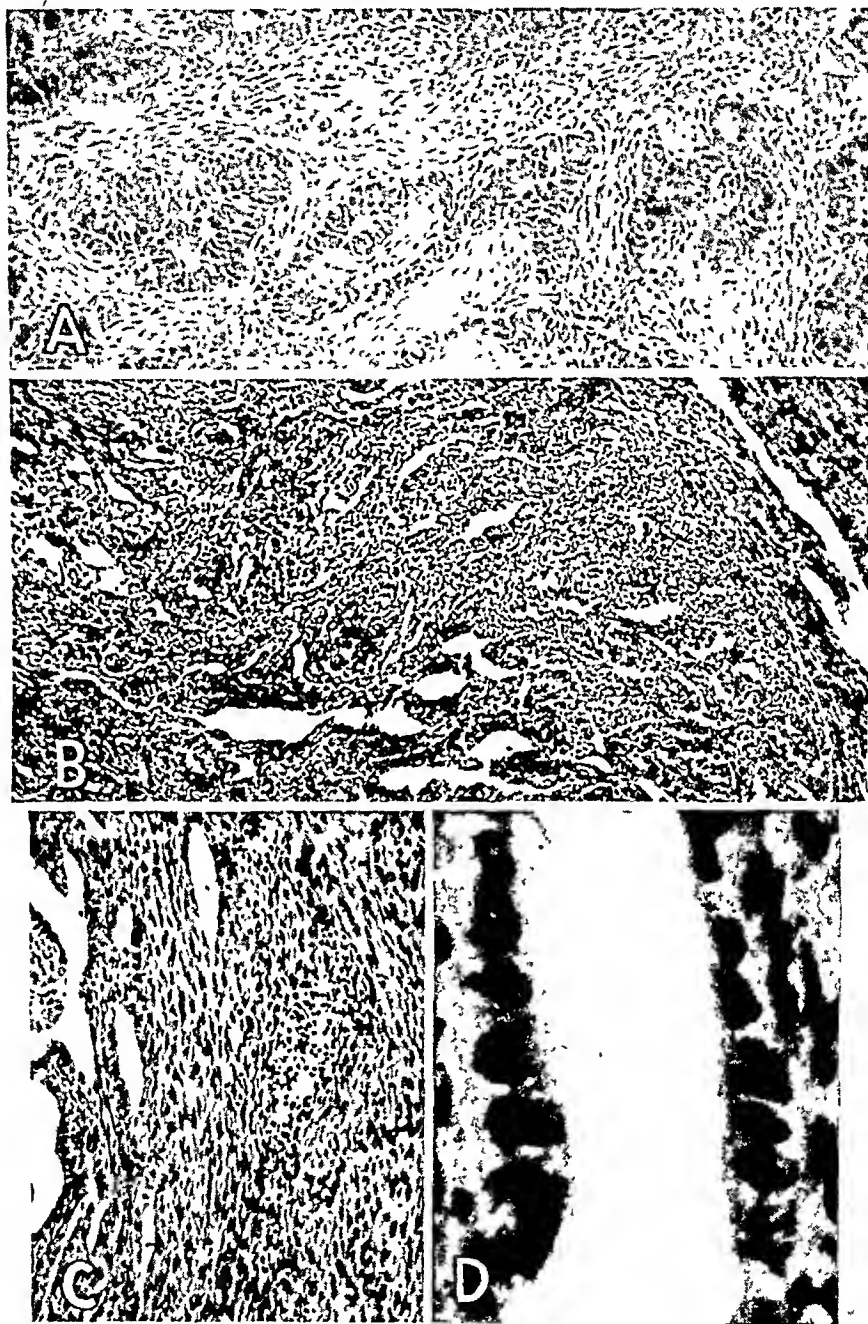


Fig. 10.—*A*, ovarian tumor with solid anastomosing ducts in fibromatous stroma and beginning formation of lumens in the thickened bud-shaped ends. The structure duplicates the fetal phase of the rete. *B*, *C* and *D*, ovarian fibroma with short, partially spindle-shaped ducts which anastomose at small angles. Note the distinct cuboidal epithelium. The shape of the ducts and the epithelium duplicate the rete ovarii.

is to be compared to the phase of solid strands which in embryonic development precedes the formation of a lumen in almost all the ducts. The relation of the two types corresponds to the relation between the trabeculated immature arrhenoblastoma with thin solid strands and the mature testicular adenoma with cordlike ducts in which distinct lumens have developed (fig. 10 A).

A case with a still younger earlier phase in the development of the epithelial constituents of the tumor has been described by me in a paper <sup>14</sup> which deals with the origin of the Brenner tumor from the rete ovarii: This case was one of multilocular serous fibrocystoma. The tumor was the size of a man's head and showed large cystic cavities containing serous fluid, lined with a high columnar, partially ciliated epithelium and separated by thick fibromatous septums, which in certain areas were as much as a thumb's width in thickness. Close to the hilus, corresponding to the location of the rete, the fibromatous stroma showed numerous islands of densely arranged cells. These cells had rather large dark nuclei and small amounts of protoplasm. They underwent transformation into epithelial elements. This phase is to be correlated with the formation of the rete from the local stroma: first by transformation of the local mesenchymal cells into solid medullary cords and later by transformation of these cords into the channels of the rete, as Fischel <sup>15</sup> has described.

Dr. Otto Saphir, of Chicago, supplied the history and the slides of a case in which the tumor presents a still greater similarity to the normal rete ovarii: A 59 year old white woman, eight years past the menopause, complained of pressure in the suprapubic region and constipation for three months. At laparotomy a large fibroma-like tumor replacing the left ovary was removed. The uterus was normal in size and contained an intramural fibroid, the size of a hazelnut. Tumor nodules were found on the serosa of the small intestine, and one in the omentum, the latter the size of an orange. The right ovary seemed to be normal in size and shape.

The specimen consisted of a massive tumor, weighing 920 Gm. and measuring 15 by 12 by 8 cm. The external surface was mottled yellow-gray, red and purple and was ragged. The tumor parenchyma was nodular and of a semifirm consistency. The sectioned surface was grayish pink with multiple hemorrhagic dots. Also present was a small similar tumor node, measuring 6 by 4.5 by 2 cm., of the same structure. Adherent to this were irregular pieces of fatty tissue (mesentery).

The microscopic sections of the ovarian tumor presented a cellular, well vascularized fibroma. In many areas the spindle-shaped cells were getting shorter and possessed a small amount of protoplasm only; the nuclei of these cells were largely hyperchromatic and of irregular, even bizarre shape. This transformation indicated the sarcomatous change which caused the intraperitoneal deposits. In the region of the hilus the fibromatous stroma included a network of cavities like channels or sinuses, which anastomosed at sharp angles and were lined with an indistinct

14. Schiller, W.: Arch. f. Gynäk. **157**:65, 1934.

15. Fischel, A.: Lehrbuch der Entwicklung des Menschen, Berlin, Julius Springer, 1929.



Fig. 11.—*A* and *B*, examples of postclimacteric rete; *C*, epithelium of *B* under high power.

single row of little cuboidal cells with pale protoplasm and lightly staining nuclei. This system of cavities gave the impression of a magnified proliferating rete ovarii (figs. 10 *B*, *C* and *D* and 11).

These 4 tumors evidently present an entity characterized by ductlike and glandlike inclusions embedded in a well developed fibroma-like stroma. They are different and have to be distinguished from the parvilocular cystoma. The differences are: first, there are no round or oval cavities but narrow strands or ducts; second, the fibromatous stroma forms a comparatively much greater part of the tumor parenchyma, and third, the mucinous secretion is not present in all cases and if present is not distinct. The ducts and glands have such small lumens that grossly, even when examined with a magnifying lens, the tumor seems to be solid and consequently in all instances has been diagnosed



Fig. 12.—Hyperplastic prostate of man 76 years old. In appearance the stroma and cysts duplicate parvilocular ovarian tumors.

as simple fibroma. Neither transition to papillomatous proliferation nor carcinomatous transformation of the epithelial part was ever observed.

The similarity of certain areas in some of the parvilocular ovarian tumors to a hyperplastic prostate is striking (fig. 12). No conclusion, so far as the histogenesis is concerned, can be drawn for the time being. The area where the prostate and the area where the gonads form are too far from each other for one to consider fetal misplacement, and there is no evidence to support a teratoid origin of the tumors.

The parvilocular adenofibroma contains no hormone-producing tissue and consequently produces no endocrine interference with the menstrual cycle. It produces no virilization of the patient. For this reason it has to be separated from the testicular adenoma, although a certain morphologic similarity cannot be overlooked, a similarity which is due



to the similarity between fetal rete and fetal germinal cords. However, in the testicular adenoma, which represents the highest degree of maturity of the arrhenoblastoma, the interstitial tissue forms thin septums only and never fibromatous masses as in the adenofibroma. The arrhenoblastoma, particularly the trabeculated type, shows adjacent to the cords groups of Leydig cells, which are missing in the adenofibroma. The localization of the ducts and glands close to the hilus, as well as the morphologic similarity, makes it probable that the tumor develops from the fetal remnants of the rete. Thus, in the general classification of ovarian neoplasms the tumor has to be placed together with the granulosa cell tumor and the fibroma in the group of ovariogenic tumors. As a name I should like to suggest "adenofibroma" in the sense that a combination of fibroma and cystoma is called fibrocystoma or cystofibroma. To indicate the narrowness of the glands the attribute "parvilocular" may be added.

#### SUMMARY

The parvilocular cystoma is an ovarian tumor characterized microscopically by small cystic cavities lined by a mucin-producing epithelium and embedded in a fibrous stroma. Papillomatous proliferation and carcinomatous transformation may be observed. The parvilocular adenofibroma presents ducts and narrow glands embedded in a well developed fibroma-like stroma: it probably originates from fetal remnants of the rete ovarii.



## FILARIAL EPIDIDYMOFUNICULITIS

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The medical departments of the Army and the Navy are emphasizing instruction in tropical medicine. The traditional concept that certain diseases are tropical, delimited geographically, is rapidly vanishing. Many diseases which are now widely disseminated originally had a restricted distribution.

World War II and modern facilities for swift travel have abolished the physical barriers that formerly isolated geographic regions, some of which have been from time immemorial the only foci of certain diseases. When American Expeditionary Force II returns home, many will have diseases unfamiliar to American physicians. New diseases will probably be introduced and spread, which the physicians of the United States must be prepared to recognize and take speedy measures to prevent.

Next to malaria, filariasis (*Wuchereria bancrofti*) is the most prevalent "tropical" disease. It is indigenous in almost all warm regions of the world, and it is extremely common in this nation's most important theaters of war in the Far East. In many of the islands of the Pacific 60 to 80 per cent of the inhabitants are infected. Like the malarial parasite, the filaria is introduced into its human host through a mosquito bite.

Filarial disease of the male genitalia is a distinct clinical condition and the most common manifestation of the infection in man. Patients with chronic swellings about the spermatic cord, testis, scrotum and groin who have been in the tropics should always be regarded as possibly filarial.

In the following cases biopsy revealed unsuspected filarial disease of the spermatic cord and epididymis.

### REPORT OF CASES

CASE 1.—A foreign Negro seaman, aged 37, a resident of St. Vincent, Cape Verde Islands, was hospitalized because of pain in the back and in the scrotum. One month previously he had injured his back aboard ship. The pain in the scrotum was of about one month's duration. The general examination showed nothing of importance. The scrotum disclosed a hard nodular lesion of the right epididymis. One examiner interpreted this to be cysts of the epididymis, some of

which were calcified. The patient was aware of the lesion and stated that it had been present for ten months. He had an evening temperature of 37.2 to 37.4 C. (98.9 to 99.3 F.). The Kolmer and Kahn tests were strongly positive.

Exploration of the right scrotal sac revealed that the spermatic cord was thickened, matted and beaded with roughly ovoid stony-hard grayish nodules, 3 mm. to 2 cm. in diameter. Here and there little pouches filled with fluid were present. The epididymis was large and stony hard and presented a coarse hobnail appearance. Small hard nodules and vascularized hard plaques were present over the surface of the testis. The nodules cut like cartilage, and many



Fig. 1.—Granulomatous obliterative endolymphangitis with beginning focal necrosis.

were largely calcified. The saccules contained turbid yellowish watery fluid. Their interior presented a strawberry-like surface.

The condition was interpreted as a neurofibromatous lesion, and the cord and testicle were removed.

Histologic examination of various levels of the spermatic cord showed marked old interstitial fibrosis, fibrotically thickened capillaries and sparse infiltrations of plasma cells and lymphocytes. The adventitia of the vas deferens was moderately thickened but not otherwise altered. The arteries showed no significant changes. The veins showed muscular hypertrophy. Here and there small sausage-shaped, ovoid and piriform calcified bodies thickly encapsulated in hyalinized connective

tissue were present. Discernible lymphatic vessels showed sclerosis and more or less old obliterative lymphangitis. Some contained refractile crystalline plaques and calcified debris. The tumor nodules present in the gross specimen were formed of whorls and interlacing strands of hyalinized fibrous tissue. They contained calcified segments of filaria. Fibrotically thickened capillaries and focal infiltrations of plasma cells were present. The epididymis was entirely replaced by fibromatous nodules having the same structure. The tunica albuginea showed focal plaques of sclerosis sparsely infiltrated by plasma cells. The parenchyma of the testis showed no pathologic changes.

Sections of the sacculs showed these to be fibrosing lymphoceles lined by fibrosing granulomatous tissue.

The diagnosis was chronic and obsolete filarial epididymofuniculitis.

The patient made an uneventful recovery.

CASE 2.—A white foreign seaman, aged 28, a native of Fort de France, Martinique, was hospitalized Oct. 31, 1941 because of a swelling in the scrotum. Two and a half years previously he had an operation for hydrocele. He was well until fourteen days prior to admission, when swelling and tenderness developed in the left side of the scrotum. Examination disclosed nothing except for the changes in the scrotum and the left inguinal region. An old operative scar and a small hard swelling were present in the left groin. Both testicles were larger than normal and firm. The left epididymis was large and tender, and its head felt like multiple small cysts. A spermatocele was suspected.

The left scrotal sac was explored. The spermatic cord was transformed into a thick, deformed, tortuous, varicose trunk irregularly adherent to the surrounding structures. It merged inseparably with the epididymis, the head of which looked like a cauliflower. The remainder presented a quilted appearance. The testis appeared normal. The spermatic cord and the epididymis were so completely involved in the inflammatory process and the blood supply was so compromised that orchidectomy and high vasectomy were done. Several days after the operation the left femoral and inguinal lymph nodes became large and tender. Two femoral nodes, 1 by 2 cm. in diameter, were removed for histologic examination. Two days later severe lymphangitis developed over the anterior surface of the thigh from the groin to the knee.

Roentgen rays of high voltage were directed to the groin and the thigh. The lymphangitis subsided, but the lymphadenitis remained unchanged, and the nodes involved were tender. The operative incisions healed uneventfully. The red and white blood cell counts and the differential blood cell picture were within normal limits. The Kolmer and Kahn tests were negative. *Plasmodium vivax* was found in thick blood smears. Repeated examinations of fresh and stained, thin and thick films of blood obtained between the hours of 10 p. m. and 2 a. m. and at other times failed to show microfilariae.

Histologic sections disclosed lesions of the lymphatic vessels in various stages of development in the spermatic cord and the epididymis. Lymphangiectasia was prominent, and the large vessels showed moderate to marked hypertrophy of their wall. Occasional lymphatics showed endothelial hyperplasia and swelling. The majority showed various degrees of epithelioid cell granulomatous endolymphangitis involving short stretches or the entire intima. In some vessels the granulomatous reaction was villiform. Occasionally the lumen was subdivided into compartments separated by delicate granulomatous septums. Many vessels showed partial granulomatous obliteration. Some showed massive obliteration with recanalization, and others showed complete granulomatous occlusion. One or more Lang-

hans giant cells were frequently seen in the granulomatous lesions, and often these were densely infiltrated by plasma cells and eosinophils. Occasionally miliary eosinophilic abscesses were present. In some lymphatic vessels the granulomatous proliferation showed foci of fibrinoid degeneration. Others showed foci of sup-



Fig. 2.—*A*, marked hypertrophic and fibrosing lymphangitis with gravid adult filariae. *B*, sclerosing lymphangitis with dead and calcifying worms.

purative thrombolympfangitis with more or less extension of the inflammation into the perilymphatic tissues. Bacteria were not demonstrable in these lesions. Some vessels showed more or less fibroplasia of their wall with lymphocyte, plasma cell and eosinophilic cell infiltration. Others showed advanced fibrohyaline thickening

with varying degrees of stenosis of the lumen. Here and there in dilated, granulating or fibrosed lymphatic vessels sections of gravid female filariae and remains of dead worms in various stages of disintegration and calcification were present. The cord, the epididymis and the adjoining tunica albuginea showed various degrees of active interstitial fibrosis, irregular edema, capillary vascularization with capillary endothelial swelling and fibrosis, areas of dense eosinophilic cell, plasma cell and lymphocyte infiltration, and islets of liposis.

The arteries showed no lesions. Some veins showed hypertrophy of their wall and minor to moderate degrees of adventitial fibrosis. The vas deferens showed no pathologic alteration. The tubules of the epididymis were dilated, and many contained spermatozoa. The testis showed some subcapsular and slight irregular interstitial lymphocyte and plasma cell infiltration.

The femoral lymph nodes showed old and active capsular fibroplasia, edema, capillary vascularization, stretches of dense lymphocyte and plasma cell infiltration, lymphangiectasia and obliterative granulomatous and fibrosing lymphangitis. The follicles were large and hyperplastic. Irregular hyperplasia of the endothelium of the sinus and obliterative granulomatous sinusitis were present. In areas there were conglomerate epithelioid granulomatous nodules with two to five Langhans giant cells. There were moderate diffuse capillary vascularization and fibrosis of the trabeculae and stroma.

The diagnosis was subchronic and subacute suppurative filarial epididymo-funiculitis with granulomatous lymphadenitis.

#### COMMENT

In the male, filariasis manifests itself most frequently in the genitalia. The basic lesion is a characteristic obliterative granulomatous endolymphangitis. As a result of the lymphatic blockage, the micro-filariae cannot enter the circulation and the parent worms die in situ. Some disintegrate and are absorbed; others calcify and are encapsulated, with the formation of hard fibromatous nodules. Frequently the earliest or the only evidence of the infection is a nodular or a cystic condition of the cord or the epididymis. Usually the patient is entirely asymptomatic. He may complain of the nodules in the scrotum or of the thickened cord, or the lesions may be detected during a routine physical examination. Involvement is frequently bilateral, with the lesions usually more advanced on one side.

In acute and subacute stages the granulomatous endolymphangitis is frequently associated with more or less suppurative inflammation. It is extremely doubtful whether bacteria play any part in the reaction.

The acute symptoms vary in severity, but pain, fever, chills and sweating are usually present. The patient usually has pain in the groin, the spermatic cord is swollen and indurated, the epididymis is swollen and very tender, and not infrequently an acute hydrocele develops. The scrotal skin may become swollen and edematous and the lymph nodes in the groin and the perilymphatic tissues acutely inflamed. The acute reaction may subside in a few hours or last

several days. The cord and the epididymis may remain indurated for several weeks. The hydrocele may be absorbed or it may persist.

The disease may become subacute, chronic and recurrent, or obsolete, with nodular or varicose changes in the spermatic cord or the epididymis as the only evidence of its occurrence.

In early stages, there is constant high eosinophilia, and microfilariae are demonstrable in the peripheral blood in a high percentage of patients. In searching for them the periodic and the nonperiodic species must be remembered. In the chronic and recurrent disease lymphatic blockage greatly reduces the chances of microfilariae reaching the blood stream, and in not more than 24 per cent of the cases of the disease in this stage are the findings positive.<sup>1</sup> Roentgenograms are useful in demonstrating calcified worms in the tissues. The parent worm and microfilariae are frequently present in the varicose lymphatics or in close proximity to them. Aspirated fluid may reveal the worms or chyle. In about 10 per cent of cases the hydrocele fluid contains microfilariae. Biopsy usually reveals the filarial nature of the lesion.

#### SUMMARY

With American Expeditionary Force II fighting in all parts of the world, and particularly with divisions in the Far Eastern theaters, where filarial infection is extremely common, attention is invited to filarial epididymofuniculitis, a frequent manifestation of filariasis. Two cases are reported.

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1. Makar, N.: J. Egyptian M. A. **21**:682, 1938.

# Case Reports

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## DISSEMINATED OSSIFICATION OF THE LUNGS

H. GIDEON WELLS, M.D., AND CHARLES E. DUNLAP, M.D., CHICAGO

Ossification of bronchial cartilages and of scars from tuberculosis or other lesions in the lungs is a common process. But diffuse forms of ossification, either ramified areas or diffusely scattered nodules, are rare. Manzini<sup>1</sup> reviewed the literature to 1938 and found but 43 cases, which he tabulated, and since that time but 2 more cases, confirmed by necropsy, have been found recorded. Strangely enough, not a single case report can be found in the English language, most of them being in German with a very few in French and Italian. Therefore it seems, desirable to put on record a typical case and to discuss it from the standpoint of pathology briefly.

The diffuse form of pulmonary ossification occurs in two forms, racemose or branching (called *veraestelte* by the Germans) and nodular circumscribed (called *tuberöse* by the Germans). A third "diffuse" form, described by Cohn and included in most German classifications, is not diffuse but localized, and probably not related to the other forms. The racemose form is by far the commonest and consists of branching spicules of true bone running in the septums of the lungs, often continuous for some distance but with isolated spicules. Usually the process is limited to certain parts of the lung and is not truly diffuse. About 35 cases of this type have been described, which occurs almost exclusively in old men, in many of the cases affecting men of quite advanced years. The best explanation for this form is that advanced by Daust,<sup>2</sup> who stated that it appeared to be merely a metaplasia due to senile alterations in perivascular connective tissue. First the vascular media degenerates; then the perivascular connective tissue swells greatly and becomes hyaline and glassy. Following this, nuclei appear in the hyaline tissue, which are short and compressed, with dendritic processes, and apparently form osteoid tissue which becomes calcified with formation of bone. He speaks of preliminary calcification leading to bone formation, but nowhere in his or other articles is there found a description of calcification as a precursor of ossification. Rather a hyaline matrix of osteoid tissue is converted into true bone by the deposition of calcium. Marrow formation is occasionally observed in this new bone, but apparently it is much more rare and sparse than that commonly found in bone resulting from calcification of tuberculous scars or ossified bronchial cartilage.

The other form, the nodular circumscribed, or *tuberöse*, is distinctly different, both anatomically and in the class of patient involved, for it

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1. Manzini, C.: Riv. di pat. e. clin. d. tuberc. **12**:145, 1938.

2. Daust, W.: Frankfurt. Ztschr. f. Path. **37**:313, 1929.

3. Janker, R.: Fortschr. a. d. Geb. d. Röntgenstrahlen **53**:260, 1936.

affects chiefly relatively young persons with mitral stenosis. Janker<sup>3</sup> reviews the 7 cases reported to 1936 in which the condition was observed at necropsy and 3 cases in which it was observed only roentgenologically, and finds that 6 patients were aged from 20 to 30, 2 from 30 to 40 and but 1 between 40 and 50, this being in marked contrast with the average age of 67 in 23 cases of the racemose form collected by Daust. Furthermore, 7 of the patients had advanced cardiac disease. Anatomically, the lesions were characterized by being small, commonly less than 0.5 cm. in diameter, discrete and scattered widely through the lungs but especially near the pleura. Since this condition is observed almost exclusively in association with mitral stenosis, one would expect it to be associated with calcium-iron incrustations, but these seem not to be a precursor of the ossification, except perhaps in the case reported by Munk,<sup>4</sup> in which calcified elastic tissue elements heavily impregnated with iron were found as well as the ossified plaques. This form should be recognized roentgenologically, and Gross<sup>5</sup> reported that after his attention was called to the condition he observed 4 cases roentgenologically but did not confirm his observations by necropsy. One striking feature was the persistence of the lesion, for in 1 case it was observed to change but little in the course of eight years.

Nodular circumscribed ossification seems to be the result of connective tissue proliferation, both interstitially and within the alveoli, and would suggest interstitial pneumonia as the forerunner were it not for the fact that in most of the cases it occurs in lungs the seat of chronic passive congestion from mitral stenosis. Histologically, the lungs show marked thickening of the alveolar walls, not only with connective and elastic tissue increase, but sometimes conspicuously an increase of nonstriated muscle fibers. There is also usually observed within the alveoli an exudate of homogeneous fibrinoid material in various stages of organization. The bone occurs as lamellas, with thin small plaques, or as larger irregular masses involving both the interstitial tissue and the intra-alveolar connective tissue. No calcification is observed to precede the ossification, but rather osteoid tissue is first formed in which calcium salts are deposited. It is to be noted that in metastatic calcification of the lungs the calcium deposits do not undergo ossification, at least as far as we have seen or can learn.

The case that we have observed comes under the heading of diffuse circumscribed, or *tuberöse*, ossification.

#### REPORT OF A CASE

A woman aged 34 had suffered from mitral stenosis since childhood, with serious decompensation and marked edema on several occasions, and had been strongly advised against pregnancy. Nevertheless she became pregnant and appeared at the hospital at the seventh month in a somewhat decompensated condition. Her urine showed a trace of albumin, but there was no evidence of toxemia. She was given rest in bed and digitalis, under which she improved and went home to remain in bed until time for a cesarean section. On the day that she returned to the hospital there developed what was diagnosed as a thrombosis of the

4. Munk, E.: J. de radiol. et d'électrol. **23**:58, 1939.

5. Gross, A.: Fortschr. a. d. Geb. d. Röntgenstrahlen **58**:33, 1938.



iliac artery. Her child was delivered by cesarean section a few days before term. The operation was uneventful, but postoperatively her chest filled with rales and her temperature rose above the highest point on a clinical thermometer. She vomited terminally and died on the day after operation. Unfortunately, no roentgenogram of the chest were made.

At necropsy there was found a high grade mitral stenosis, the orifice barely admitting the tip of the little finger. There was a solitary red vegetation, 1 mm. in diameter, near the valve margin. Besides the findings of a recent cesarean section, a recent small mural thrombus in the abdominal aorta and acute passive congestion of the liver, the only abnormality was in the lungs. These showed acute bronchopneumonia and were dark purple. Lying in the visceral pleura over all lobes were minute flat plaques of ossification, averaging 1 mm. in diameter. Occasional calcific deposits similar to those in the pleura were present throughout all the lobes. No fibrosis was apparent about these areas of ossification. The pulmonary vessels were thick walled but not calcified, and there was no calcification in the peribronchial nodes. No brown pigmentation was discernible; if present, it was obscured by the congestion. No other organ showed any calcification whatever. On examination of roentgenograms made of the lungs after the necropsy there were found at least 200 opaque flecks, widely distributed through both lungs but more in the lower lobes than elsewhere. None of the flecks was over 4 mm. in diameter (fig. 1).

Microscopically, the lungs presented diffuse acute hemorrhagic bronchopneumonia, with minimum deposition of hemosiderin pigment, superimposed on an older process. The alveolar walls were generally but not uniformly thickened by fibrous tissue formation, in some places by nonstriated muscle fibers and in places by a hyaline material apparently derived from the increased connective and elastic tissue. The pulmonary arteries were thickened and in places hyalinized. It was surprising to find so much thickening of the alveolar walls with so little iron pigmentation. Many of the alveoli contained marginal deposits of hyalinized exudate, which exhibited various stages of organization and in some places had the appearance of osteoid tissue. Nowhere was calcification observed independent of bone formation.

The bone appeared in two forms—as flat plaques in the pleura (fig. 2) and as branching deposits within the lung proper (fig. 3). The latter seemed to be both interstitial and intra-alveolar. In either case the bone consisted of lamellas of bone with abundant bone corpuscles but as far as could be found, no marrow formation.

The fibrosis of the lung was different from that seen ordinarily in chronic passive congestion. First, there was the extremely sparse deposition of iron pigment and the rather focal distribution of the connective tissue increase, for there were areas that seemed relatively free from it. Second, there were organizing fibrinoid deposits within the alveoli and thickening and hyalinization of the interstitial connective tissue. The appearance was that of progressive chronic interstitial pneumonitis with ossification. Were it not that in nearly all the cases of ossification of this type there has been associated advanced chronic cardiac decompensation, it would seem as if the decompensation had nothing to do with it. However, such ossification has not been observed in uncomplicated cases of chronic interstitial pneumonitis and very rarely in cases of chronic pulmonary congestion, even when this was associated



Fig. 1.—Roentgenogram of the right lung showing disseminated ossified flecks throughout, although chiefly in the lower lobe. The left lung showed practically the same picture.

with pneumonitis. The possibility remains that the conditions favorable for ossification in chronic interstitial pneumonia are present only when pulmonary congestion is added to the picture. There is considerable evidence that venous stasis or general circulatory impairment favors bone formation provided that other conditions conducive to ossification are present.<sup>6</sup>

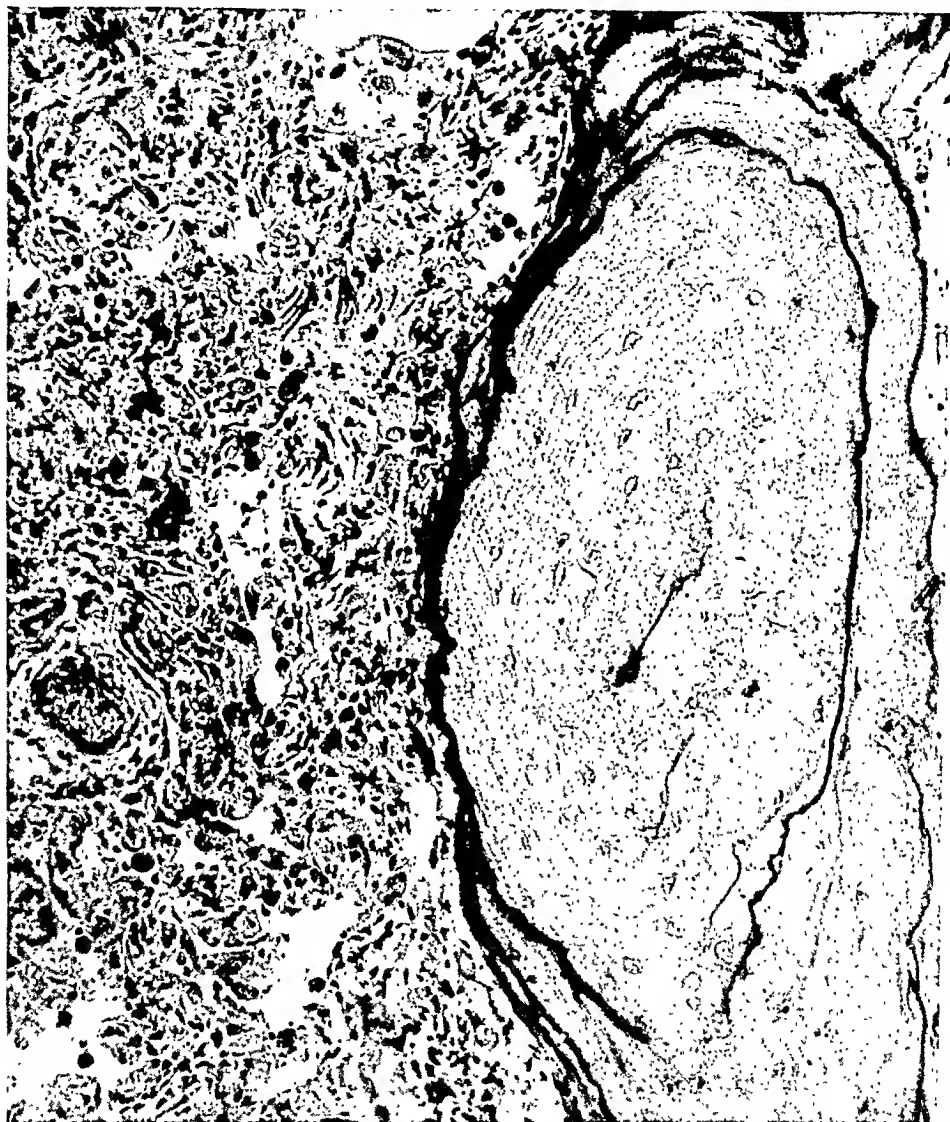


Fig. 2.—Ossified area beneath the pleura, without marrow formation ( $\times 155$ ). The underlying lung tissue shows chronic interstitial pneumonitis.

The findings in roentgen ray pneumonitis are remarkably similar to those seen in this case, especially the hyaline intra-alveolar membrane,<sup>7</sup> but in none of the human cases described in the literature is there mention

6. Roome, N. W., and McMaster, P. E.: *Arch. Surg.* **29**:54, 1934. Asami, G., and Dock, W.: *J. Exper. Med.* **32**:745, 1920.

7. Warren, S., and Spencer, J.: *Am. J. Roentgenol.* **43**:682, 1940.

of ossification occurring in this condition. 'On the other hand, roentgen ray pneumonitis experimentally produced in rabbits by Engelstad<sup>8</sup> led to ossification quite similar to that observed with mitral stenosis. He said, "At least half the animals (whose lungs were) examined two months or more after irradiation showed more or less widespread ossification in the form of small disseminated particles of bone," and his photomicrographs

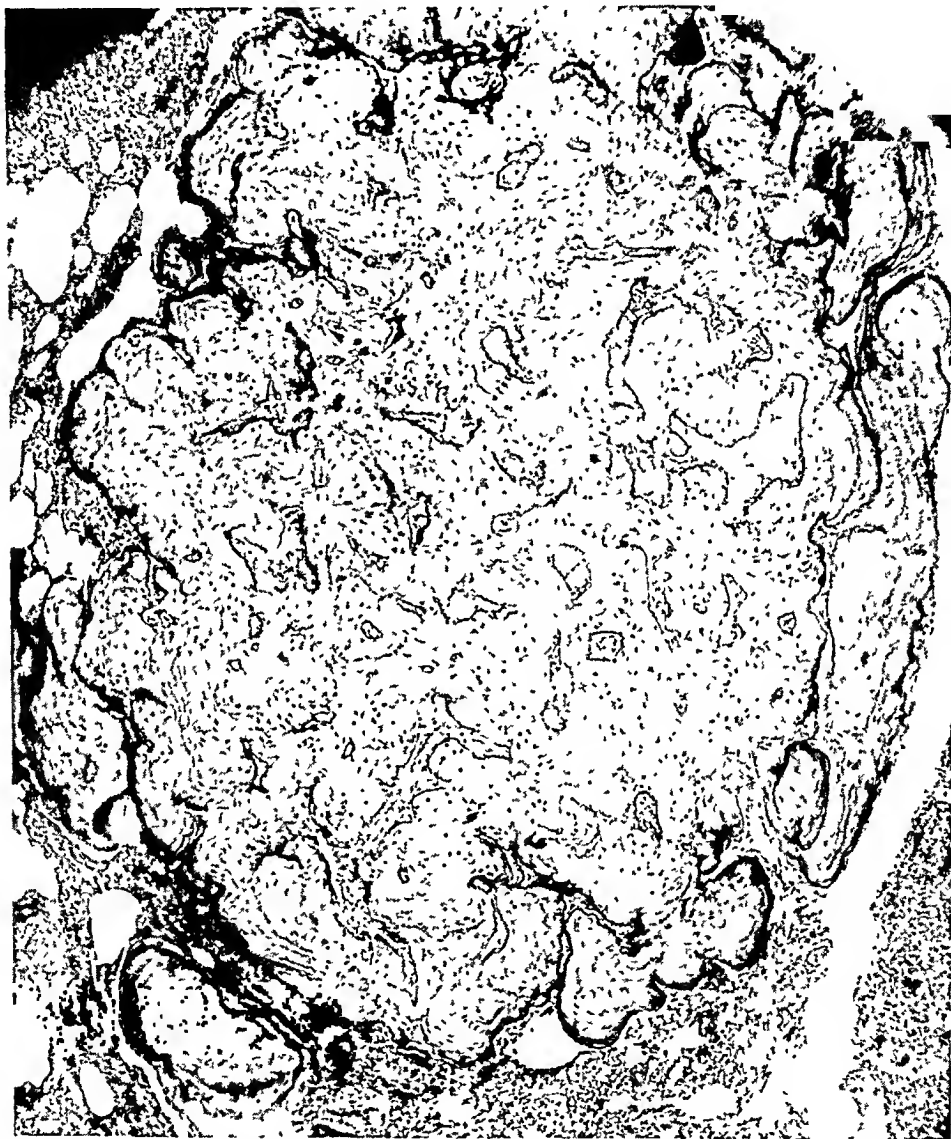


Fig. 3.—Area of branching ossification within the lung proper ( $\times 45$ ). Note the absence of marrow formation or of calcification independent of ossification.

resemble the human material in the cases of ossification associated with mitral stenosis. The susceptibility of the rabbit to calcification is well known<sup>9</sup> and is explained by the high blood calcium, Bourne and Camp-

8. Engelstad, R. B.: *Acta radiol.*, 1934, supp. 19.

9. Wells, H. G.; Holmes, H. F., and Henry, G. R.: *J. M. Research* **20**:373, 1911.

bell<sup>10</sup> having found the normal figure to be from 12.4 to 18.6 mg. per hundred cubic centimeters. We cannot find an increase in calcium to be a constant occurrence in chronic passive congestion of the lungs, but it might be that in occasional cases the rise in carbon dioxide is responsible for an increase in blood calcium.

#### SUMMARY

About 45 cases of diffuse ossification of the lung unassociated with tuberculous scars have been reported. In the majority the ossifying process was of a branching type. The patients were almost exclusively aged men. About 7 verified cases of a circumscribed, nodular type, characterized by formation of discrete small flecks of bone throughout the lung, have been described. The patients were relatively young persons with mitral stenosis. To this group of cases we have added a typical example.

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Harvard Medical School.

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10. Bourne, M. C., and Campbell, D. A.: *Biochem. J.* **26**:183, 1932.

## CANDIDA ALBICANS INFECTION CONFUSED WITH TUBERCULOSIS

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Although *Candida* (*Monilia*) not infrequently attacks various tissues in the human body, such as the skin, the mucous membranes and the respiratory tract, involvement of the central nervous system is extremely rare. A careful search of the literature reveals only a single previously reported case in which the fungus was definitely studied and identified as *Monilia albicans*. This was reported by Smith and Sano<sup>1</sup> in 1933. These authors were also unable to find reference to any previously reported case. The case to be presented would therefore appear to be the second in the literature. Other features of interest are the accompanying stomatitis, laryngitis and ophthalmitis due to *Candida*. During life the lesions were confused with those of tuberculosis.

Meningitis due to pathogenic fungi is not a rarity. Since Stoddard and Cutler<sup>2</sup> reported 4 cases of meningitis due to *Torula histolytica*, there have appeared scattered reports from various regions of the United States and other parts of the world, such as those of Pierson,<sup>3</sup> Evans,<sup>4</sup> Sheppe,<sup>5</sup> Shapiro and Neal,<sup>6</sup> Levin<sup>7</sup> and Ball.<sup>8</sup> Freeman<sup>9</sup> mentioned a case of meningitis caused by *Saccharomyces* and reviewed 19 other cases of various causation. Anderson<sup>10</sup> reported 4 cases of mycosis of the brain, 1 of which he attributed to *Saccharomyces*, 2 to *Torula* and 1 to *Coccidioides*. Rewbridge, Dodge and Ayers,<sup>11</sup> reported a case due to *Endomyces capsulatus* (new species). Blastomycosis of the brain has been reported by Swift and Bull,<sup>12</sup> Badham,<sup>13</sup> Gaspar,<sup>14</sup>

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From the Department of Pathology of the University of Rochester School of Medicine and Dentistry.

1. Smith, L. W., and Sano, M. E.: *J. Infect. Dis.* **53**:187, 1933.
2. Stoddard, J. L., and Cutler, E. C.: *Torula Infection in Man*, Monograph 6, Rockefeller Institute for Medical Research, 1916.
3. Pierson, P. H.: *J. A. M. A.* **69**:2179, 1917.
4. Evans, N.: *California State J. Med.* **20**:383, 1922.
5. Sheppe, W. W.: *Am. J. M. Sc.* **167**:91, 1924.
6. Shapiro, L. L., and Neal, J. B.: *J. A. M. A.* **81**:212, 1923.
7. Levin, E. A.: *Arch. Int. Med.* **59**:667, 1937.
8. Ball, H. A.: *California & West. Med.* **32**:338, 1930.
9. Freeman, W.: *Ann. Int. Med.* **6**:595, 1932; *J. f. Psychol. u. Neurol.* **43**:231, 1931.
10. Anderson, G. C.: *Arch. Surg.* **42**:379, 1941.
11. Rewbridge, A. G.; Dodge, C. W., and Ayers, T. T.: *Am. J. Path.* **5**:349, 1929.
12. Swift, H., and Bull, L. B.: *M. J. Australia* **2**:265, 1917.
13. Badham, C.: *M. J. Australia* **2**:385, 1922. Cabot Case 25292, *New England J. Med.* **221**:111, 1939.
14. Gaspar, I.: *Arch. Neurol. & Psychiat.* **22**:475, 1929.

Moore<sup>15</sup> and Wilhelmj.<sup>16</sup> Coccidioides infection of the spinal cord was reported by Rand.<sup>17</sup> Hyslop<sup>18</sup> reported a case of sporotrichotic meningitis. Actinomycosis of the meninges has been reported by Bell<sup>19</sup> and Moersch.<sup>20</sup> There is a recent report of endocarditis due to *Candida* by Wikler and associates.<sup>21</sup> In addition, there are occasional scattered reports of yeast-caused meningitis in which the fungus was so inadequately studied as to render the report incomplete.

#### REPORT OF A CASE

The patient was a 30 year old white man. The history dates back to 1933, at which time the patient noted small white spots in the mouth and on the tongue. Following tonsillectomy in 1934 these became red, bleeding and painful, particularly after he had eaten hard, dry foods. The condition became progressively worse, and in August 1936 he was referred to the State Institute for the Study of Malignant Disease, in Buffalo. A biopsy specimen was taken from the mouth, and the following report was rendered: "This is tuberculosis of the submucous area. The surface epithelium is markedly hyperplastic and thickened. Typical tubercles are found below it."

In September 1936 he was seen in the outpatient department of the Mount Morris Tuberculosis Hospital, Mount Morris, N. Y., at which time he complained of the oral lesions and of an occasional cough, sore throat, sense of fulness in the throat and easy fatigability. Examination revealed nothing except for the lesion of the mouth. This was described as a chronic granulating process involving the buccal surfaces of the mouth, the inner surfaces of the lips and the surfaces of hard palate, soft palate, uvula, anterior pillars and epiglottis. The surfaces showed heaping up, induration, fissuring and a fine granularity with some injection but no bleeding. The roentgen picture of the chest showed no pathologic change. Sputum revealed no acid-fast organisms. A serum complement fixation test for tuberculosis was negative. A tuberculin test with 0.005 mg. of purified protein derivative was positive. The Wassermann reaction of the blood was negative. The patient was given a course of generalized treatment with ultraviolet rays. However, because of the development of iritis and uveitis, this therapy was discontinued until such time as the ocular condition improved.

The patient was then admitted to the eye service of the Strong Memorial Hospital, in Rochester, N. Y., in February 1937. At that time he stated that one month after the start of the ultraviolet ray treatment he noticed spots and blurring of vision in the right eye, followed rapidly by severe pain and redness. At the time of admission there was total loss of vision in the right eye.

The past history was essentially without bearing on his condition. He was working in a restaurant when the oral lesions first developed, and there were no contacts with persons known to be tuberculous.

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15. Moore, J. T.: Surg., Gynec. & Obst. **31**:590, 1920.

16. Wilhelmj, C. M.: Am. J. M. Sc. **169**:712, 1925.

17. Rand, C. W.: Arch. Neurol. & Psychiat. **23**:502, 1930.

18. Hyslop, G. E.; Heal, J. B.; Kraus, W. M., and Hillman, O.: Am. J. M. Sc. **172**:726, 1926.

19. Bell, H. H.: J. Infect. Dis. **30**:99, 1922.

20. Moersch, F. P.: Arch. Neurol. & Psychiat. **7**:745, 1922.

21. Wikler, A.; Williams, E. G.; Douglas, E. D., and Emmons, C. W.: J. A. M. A. **119**:333, 1942.

The following positive physical findings were recorded: There were enlarged, nontender cervical nodes. The left eye was normal except for internal strabismus. The right eye showed chemosis and edema of the bulbar and palpebral conjunctiva. The eyeball was somewhat shrunken, soft and tender. The cornea was opaque, with vascularization and small hemorrhagic areas in the region of the pupil. The lips were dry and fissured, and at the corners of the mouth there were deep fissures with heaped up, coarsely granular edges which merged into the buccal surfaces presenting the same thick, gray, granular appearance. The dorsum of the tongue was similar in appearance. There were a few hemorrhagic areas.

The clinical impressions were: panophthalmitis on the right, probably tuberculous; tuberculosis of the mouth.

The right eye was enucleated. Sections were interpreted as showing an acute and chronic granulomatous lesion. The inflammatory reaction involved all coats. Acid-fast stains of the sections were negative for tubercle bacilli.

A week later a biopsy specimen was taken from the inner surface of the upper lip. This was also reported as showing nonspecific granuloma. Because the patient complained of hoarseness, he was seen by a consultant on diseases of the nose and throat. The epiglottis showed the same type of lesions as the mouth. The arytenoids and ventricular bands were swollen and slightly red. The impression was that the lesions were probably tuberculous.

Laboratory tests were essentially negative. Direct smears and inoculations of guinea pigs with material from the mouth were negative for acid-fast organisms. Sputum was also free from such organisms on smear and on inoculation of guinea pigs.

The man was discharged and was seen at long intervals in the ophthalmologic outpatient department for glasses and a routine follow-up. From March 1937 to September 1941 his condition was apparently unchanged.

Nov. 27, 1941, he was brought in stuporous and lethargic and unable to give a clear history. The available information indicated that he had suffered from very severe frontal headaches for the previous two months. These were worse at night. For the previous two weeks there had been marked lethargy, and the patient was unable to respond to spoken words. He was getting weaker rapidly and had fever and generalized muscular pains.

His temperature was 38.2 C. (100.8 F.), pulse rate 86 and respiratory rate 24; his blood pressure was 106 systolic and 70 diastolic. The following pertinent findings were noted: The patient was a 30 year old man, was very lethargic and markedly emaciated, appearing acutely and chronically ill. There was no generalized adenopathy. The teeth were foul and in bad repair. The tongue was foul and dry, covered with whitish exudate and deeply fissured. The entire buccal mucosa also was covered by a thick cheeselike material. The patient was drowsy and lethargic, the mentality was clouded, and the neck was questionably stiff. The left pupil was fixed and did not react to light. The left fundus showed choking of the disk. Other cranial nerves were intact. There were involuntary muscular contractions and fibrillary twitchings of the extremities. The deep reflexes were all absent. The Babinski reflex was equivocal.

The Wassermann reaction of the blood was negative. The red blood cell count was 4,950,000. The hemoglobin level was 15.0 Gm. The white cell count was 11,600 (neutrophils 83 per cent, lymphocytes 15 per cent, monocytes 1 per cent, eosinophils 1 per cent). The urine was normal. The spinal fluid showed an initial pressure of 250 mm. of water and a final pressure of 200 mm. The Pandy test was positive, and there were 117 cells without and 109 with acetic acid. Most of the cells in the spinal fluid were described as mononuclears. The spinal fluid



sugar was too low to read. The spinal fluid protein was 195 mg. per hundred cubic centimeters, the colloid gold curve was 2555554432, the Wassermann reaction was negative, and a smear was negative for acid-fast organisms. Examination of the spinal fluid four days later showed 122 cells, sugar too low to read, protein 150 mg. and chlorides 114 milliequivalents. Two days later the spinal fluid pressure rose to 400 mm. Spinal fluid cultures and smears were negative. A guinea pig inoculated with the fluid showed no tuberculosis. Repeated smears of exudate from the mouth were negative for acid-fast organisms.

The temperature, which on admission was 39 C. (102.2 F.), dropped to normal in the next five days, and then began to rise gradually until terminally it measured 41 C. (105.8 F.). No therapy was given other than supportive measures. The patient remained somewhat somnolent and stuporous but responded to painful and auditory stimuli. He would not eat and was maintained on parenteral fluids and dextrose. The neurologic picture remained unchanged. He became more and more lethargic and less responsive to stimuli. Terminally, he was comatose and extremely debilitated, and he died on Dec. 19, 1941, after a stay in the hospital of twenty-three days and a total illness of approximately eighty-three days.

The diagnosis at discharge was questionable tuberculous meningoencephalitis.

*Autopsy.*—The anatomic diagnosis was: chronic stomatitis, glossitis and laryngitis due to *Candida* (*Monilia*) *albicans*; meningitis and ependymitis due to *C. albicans*; granulomatous endarteritis of the superior cerebellar artery; old enucleation of the right eye (ophthalmitis due to *C. albicans*); bronchopneumonia; emaciation.

A complete autopsy was done, and all organs were carefully studied, but only those showing gross pathologic change are mentioned here.

The body was that of an extremely emaciated 30 year old white man. The right eye was absent. The lower lip had a deep ulcerated surface which extended into the mouth and was bright red, slightly granular, dry and sharply demarcated. All surfaces of the mouth were thick and granular and covered with abundant gray exudate. No cervical glands were palpated.

The lungs weighed 420 Gm. each. Both lower lobes were reddish brown and on section were moist. The cut section presented a few scattered small granular areas which were slightly firmer in consistency, grayish and bulging.

The trachea and the larynx were normal in their external configuration. When the larynx was opened, the mucosa appeared pink and glistening except in the region of the left vocal fold and ventricle. In this region the surface was granular and gray. There were no areas of ulceration, although in the posterior angle of the ventricular fold the mucosa appeared soft, edematous and reddish brown. The granular areas extended up into both ventricular appendixes and onto the posterior surface of the epiglottis. The tongue showed a markedly thickened and irregular surface, which was nodular, gray and dry. There was no gross ulceration.

The brain weighed 1,370 Gm. The dura was normal. The venous sinuses were normal. At the tip of the left parietal lobe was one small area in the pia-arachnoid which was very finely granular and grayish, consisting of many minute glistening sandy nodules which were slightly opaque. Over the right parietal lobe were seen a few small sandy nodules presenting the same appearance. These small tubercle-like structures measured only a fraction of a millimeter in diameter and were distributed about the pial vessels. The base of the brain presented a thick, grayish yellow, stringy exudate over the pons and peduncles, extending into the fissures between the temporal and frontal lobes and encircling the brain stem posteriorly. In this thick exudate were seen a few small raised tubercle-like nodules. The exudate did not involve the superior aspect of the cerebral hemispheres.

The brain was cut after fixation. The cortex was everywhere of normal thickness and showed no abnormal areas. The white substance presented no areas of softening. The lateral ventricles were of normal size, but along their entire length the ependymal surface was covered with thick, irregular nodular masses which

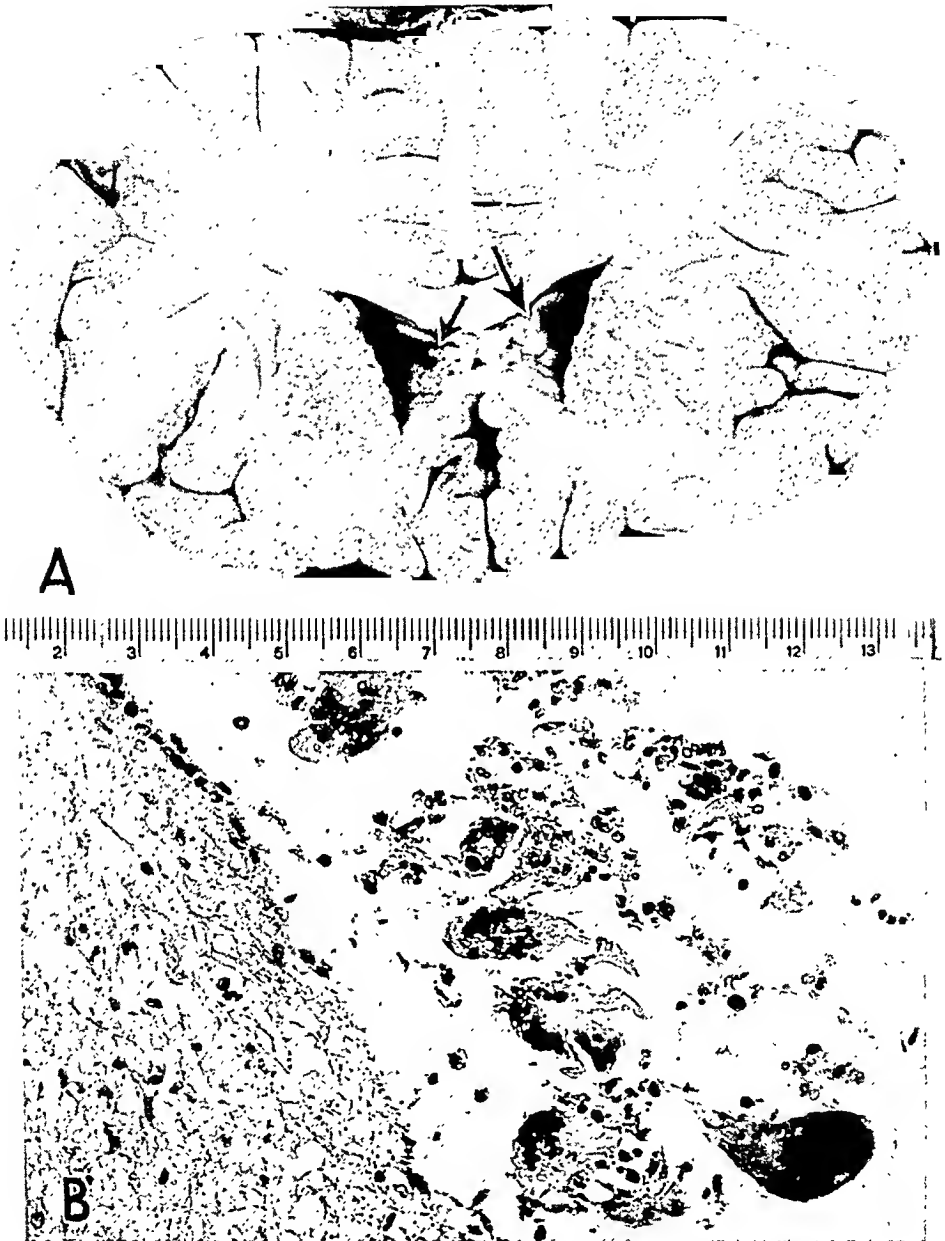


Fig. 1.—*A*, coronal section of the brain through the anterior portions of the lateral ventricles. The thick masses of *Candida* (*Monilia*) proliferating on the ependymal surfaces can be seen as glistening nodules projecting into the lumen. All ventricles showed this type of involvement. *B*, material from the lateral ventricle. This illustrates the large multinucleated giant cells seen in all portions of the exudate. Blastospores can be seen in at least two of the giant cells. This is an important feature of fungous lesions and may be useful in differentiating them from the lesions of tuberculosis. Gram stain;  $\times 210$ .

projected into the cavity. These were grayish white, glistening and translucent and seemed to be firmly attached to the surface although they were, of themselves, friable in consistency. The exudate measured about 8 mm. at its greatest thickness. The third ventricle was slightly dilated, and the ependymal surface was covered with the same kind of granular translucent exudate. The pineal gland appeared large and cystic, measuring 8 by 5 mm. The aqueduct was normal in size but occluded by the same material as that seen in the ventricles. The fourth ventricle showed the same process to a less degree. The medulla and the cerebellum were normal.

*Histologic Studies.*—Sections of all organs were taken routinely. Only the significant ones are described. All blocks were fixed in Zenker's formaldehyde solution<sup>22</sup> and embedded in paraffin.

The lungs revealed marked congestion with extravasated blood in the alveoli. There were scattered patches of bronchopneumonia. No epithelioid cells, caseation or tubercle formation was seen. The routine stains showed no fungous elements. Many clumps of bacteria were present. Special stains were negative for fungi.

Sections of the tongue and of the larynx were studied with the routine hematoxylin-eosin stain, Mallory's phloxine-methylene blue stain, MacCallum's modification of Goodpasture's Gram stain, and a stain for acid-fast organisms. The sections stained with the routine hematoxylin and eosin showed the lesions to be granulomatous in character. There was ulceration of the epithelium, with marked round cell infiltration, tubercle formation and the presence of many epithelioid cells and giant cells. A few mycelial threads could be identified, although they stained faintly. These were present in the ulcerated areas as well as in the deep tissues. In the larynx the tubercles were composed of a necrotic center and a ring of numerous multinucleated giant cells. In the caseous area a few mycelial threads were identified. There was hyperplasia of the epithelium where this was not ulcerated. The round cell infiltration was most marked in the areas of ulceration, but was also present throughout the deeper tissues (fig. 2 B).

The Gram stain brought out the fungous elements so that they could be identified with ease. They were present not only in the ulcerated areas but also in the areas of deep necrosis and in the centers of the tubercles. Both mycelial threads and blastospores could be identified. The mycelia were typical and were seen as slender rods a few microns thick, the walls of which stained darkly and had an internal structure which varied somewhat (fig. 2 A). Most typical were condensations of gram-positive material which gave them an irregularly segmented appearance and which outlined the clear oval spaces, giving the mycelia a beaded appearance. These clear spaces stained with the usual fat stains. The blastospores were easily identified when they occurred in clusters, but this formation was not usual, and the individual cells were difficult to find. They appeared as small ovoid cells about 4 microns in diameter, staining darkly gram-positive and showing a thin, doubly refractile capsule which took the counterstain poorly.

The phloxine-methylene blue stain made the fungous elements well visible but was less useful as a differential stain because, although it allowed ready identification of the mycelia, the blastospores were not easily differentiated from lymphocytes. The stain for acid-fast organisms showed that the mycelia were not acid fast, and no acid-fast organisms were found.

In view of the findings in the autopsy sections, the biopsy specimens taken from the mouth on Feb. 4, 1937 were recut and stained with the differential stains

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22. This is Zenker's stock solution with addition of solution of formaldehyde.

already described. The hematoxylin-eosin stain showed a granulomatous lesion with tubercle formation and both giant cells and epithelioid cells. The Gram stain revealed typical fungous elements in the deep tissues and in the areas of caseation.

The pathologic picture in the brain sections related to the meningeal and the ependymal reactions.

The basilar meninges showed extensive involvement. They were thickened and presented a thick exudate of lymphocytes, with scattered plasma cells, monocytes

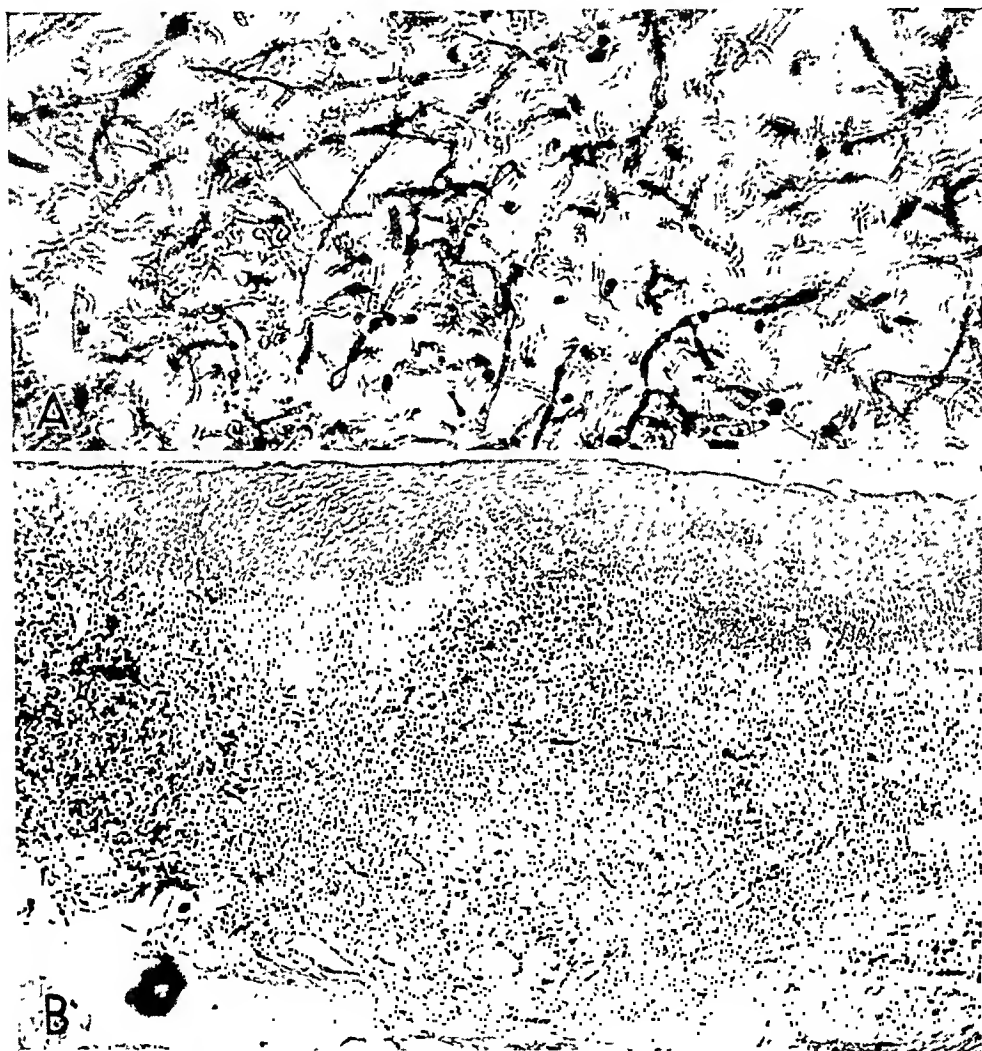


Fig. 3.—*A*, high power view of a large field of mycelia from one of the brain sections. The branching and the typical segmented and beaded appearance are characteristic. The clear vacuoles in the mycelia are also typical. Blastospores are present but are not easily identified. Gram stain;  $\times 430$ . *B*, section from the larynx showing the granulomatous appearance of the lesion. There is marked round cell infiltration throughout, and deep in the section can be seen a large tubercle. In the center of the necrosis slender gram-positive mycelia can be identified even under this magnification. Gram stain;  $\times 60$ .

and large phagocytic cells. Only an occasional polymorphonuclear leukocyte was seen. The meningeal reaction was more intense in the deep recesses of the sulci,

and in several areas large masses of mycelia were seen. Many large giant cells were seen around such areas, and many round cells were present about the margins. With special stains, the mycelia presented the typical appearance already described. Some of the cells interpreted as being lymphocytes in the sections stained with hematoxylin and eosin were seen to be typical blastospores.

The ependymal reaction was striking and unusual. Sections through the ventricles showed that the large translucent grayish white masses seen grossly were actually huge collections of fungous elements proliferating within the ventricles (fig. 1A). One of these masses was almost 1 cm. in diameter. The ependymal surface was replaced by the granulomatous tissue composed of giant cells and chronic inflammatory cells. The giant cells occasionally contained clear ovoid areas the size of one of the nuclei, and with the Gram stain these were shown to be ingested blastospores (fig. 1B). The mycelia presented a typical appearance. Blastospores were not as numerous as mycelial threads, although they were also present in large numbers.

The third and fourth ventricles were almost completely occluded by masses of mycelia. The pineal body was seen completely surrounded by mycelia and showed calcification and degeneration. Its center was cystic and was filled with a clear pink-staining colloid-like material.

The vascular system of the brain had been involved in the process. There was perivascular infiltration of the smaller vessels in the brain substance, and the cells were all mononuclears. The superior cerebellar artery was seen in cross section between the cerebellum and the pons and presented an unusual picture. The lumen was markedly narrowed by very marked thickening and proliferation of the intima. This showed degeneration of its fibers, vascularity and infiltration by lymphocytes, monocytes and plasma cells, with a large number of epithelioid cells. At the junction of the intima and media there was a separation of the two coats, and in the outer intima were many giant cells with numerous nuclei. Scattered among them were a few mycelial threads. The adventitia showed moderate mononuclear infiltration and a thick, markedly infiltrated zone which formed a complete collar around the artery. Here, again, there were great numbers of round cells, and both mycelia and blastospores were found. Other arteries showed marked perivascular reaction, but the intima was not involved.

The cerebellum showed one area of degeneration of the granular layer with loss of cells. There was no glial reaction. The Purkinje cells appeared to have been spared.

The right eye, which was enucleated in 1937, was restudied, and special stains were used. The appearance with the routine stains has been described elsewhere. The differential stains showed that in the main bulk of the exudate and necrotic tissue obliterating the posterior chamber and vitreous there was a large aggregate of typical mycelia.

*Isolation and Identification of the Fungus.*—The basilar exudate from the brain was sampled by sterile technic and streaked on standard Sabouraud agar plates. Direct smears prepared with acid-fast stains were found to be negative for acid-fast organisms.

The Sabouraud agar plates showed pure colonies, which appeared after standard incubation for twenty-four hours at 37.0 C. At first these colonies were very small, dull, grayish white and flat with smooth surfaces. Later they coalesced to form large creamy colonies with a fringe of mycelia around the margin growing radially into the medium. Among old cultures the larger colonies showed raised and puckered central areas. Smear preparations from young colonies showed gram-positive blastospores and few mycelia. The spores were gram positive, about 4 or 5

microns in diameter and oval and had thin, doubly refractile capsules. The mycelia showed occasional branching with terminal spore formation.

Young colonies from the Sabouraud agar plates were subcultured on fresh Sabouraud agar plates and on blood agar plates. On blood agar pure colonies were isolated, which appeared after forty-eight hours at 37.0 C. These were small uniform round grayish colonies with a smooth surface and edge. Further incubation produced no changes other than growth in size. The subcultures on Sabouraud agar plates showed no change in colony characteristics or in the appearance of the smears prepared from them.

Material from the growth on the original Sabouraud agar plates was suspended in saline solution and injected intraperitoneally into white mice. The mice remained alive and well for many weeks after the injection of the suspension.

On the basis of these preliminary studies it was decided that the etiologic agent was a fungus, and that this belonged in the genus *Candida* (*Monilia*).

Dr. N. F. Conant made the final identification. A culture was sent to him, and by following the procedure advocated by Martin and co-workers<sup>23</sup> he identified the fungus as *Candida albicans*. Details of the method will be found in the references cited, but briefly the identification includes the character of the growth on various mediums, fermentation of sugars and pathogenicity tests. This organism is pathogenic for rabbits.

#### COMMENT

This case presents many features of interest both to the pathologist and to the clinician. Clinically, the diagnosis of tuberculous meningitis was made. This was a likely diagnosis in view of the reported tuberculosis of the mouth, although biopsy specimens were always reported from this laboratory as showing nonspecific granuloma. At no time were acid-fast organisms demonstrated in the exudate from the mouth, in biopsy sections or in the spinal fluid. The cells in the spinal fluid were described as mononuclear cells, and it is possible that some of these may have been yeast cells. In the fresh state, and in the absence of a suspicion of any fungus, yeast cells may easily be mistaken for small lymphocytes if no attention is paid to the distinctive doubly refractile capsule. This point has been stressed by many writers on fungous diseases. A careful search must be made for fungous elements if the correct diagnosis is to be made.

Other laboratory findings are not helpful in making the diagnosis. The spinal fluid sugar in this case was always reported as too low to be read, and this finding supported the diagnosis of tuberculous meningitis. It is of interest to note the findings in other cases. Smith and Sano<sup>1</sup> did not give spinal fluid sugar levels in their case. They reported the ependymal surfaces as clear. Freeman<sup>9</sup> reported on 19 cases of fungous meningitis but stated only that "chemical studies on spinal fluid are negative." In the case of sporotrichotic meningitis reported by Hyslop and associates<sup>18</sup> the values for spinal fluid sugar ranged from 42 to 88 mg. per hundred cubic centimeters. Anderson<sup>10</sup> reported 4 cases; in 2 the spinal fluid sugar amounted to 5 to 10 mg. per hundred cubic centimeters; in 1 instance the value was not reported,

23. (a) Martin, D. S.; Jones, C. P.; Yao, K. F., and Lee, L. E., Jr.: *J. Bact.* **34**:99, 1937. (b) Martin, D. S., and Jones, C. P.: *ibid.* **39**:609, 1940.

and in the last it was 93 mg. In the case reported by Rewbridge and associates<sup>11</sup> the spinal fluid sugar amounted to 36 mg. Therefore, the sugar level may be reduced moderately or markedly, but no differential information can be obtained.

Candida infections of the mouth have been reported many times, as in the papers by Zeisler,<sup>24</sup> Engman and Weiss,<sup>25</sup> Frost and co-workers<sup>26</sup> and Smith.<sup>27</sup> They are common in childhood and are frequently seen in old people with chronic debilitating diseases. Their importance is that in the older age group they may be mistaken for tuberculosis, as in the case reported here, or for some other type of granuloma. The pathologic picture is that of granuloma, and in such cases the pathologist should attempt to demonstrate a specific etiologic agent by means of acid-fast stains, cultures, Gram stains and serologic tests. Before the diagnosis of nonspecific granuloma is made, fungous infection should be ruled out by means of differential stains.

The same applies to laryngeal lesions, although fungous infections in this region are not common. Clerf and Bucher<sup>28</sup> reported 3 cases of laryngeal moniliasis and collected 6 more from the literature. Here, again, the fungous lesion must be distinguished from other types of granuloma.

Ophthalmitis of fungous origin is extremely rare, and there has probably been no previous report of an infection due to Candida. However, because the literature is not recent, the nomenclature used for the implicated fungi is very confused, and it is impossible to be sure.

The pathologic appearance of fungous lesions is interesting. A granuloma is represented, and the lesion may be mistaken for tuberculosis by expert pathologists. Giant cells are numerous, probably more numerous than in tuberculosis, and definitely more numerous than in the usual syphilitic lesion. They often contain ingested blastospores and mycelial elements, which is an important differential point. Tubercle formation may be typical, and necrosis may be a feature. Without recognizing the fungous elements by special stains, the lesions may easily pass for tuberculosis. Candida spores are difficult to identify, even with special stains, but some of the other fungi, such as *Coccidioides* and *Blastomyces*, are more easily recognized because the spores are larger and have a thick doubly refractile capsule.

The diagnosis of a gross specimen is equally difficult. The distribution of the meningitis in this case was like that typically seen in tuberculosis, and the definite diagnosis hinged on isolation of the fungus by cultural methods and on finding the fungous elements in the histologic sections.

Classification of pathogenic fungi is a matter for experts in that field, and in this case I have been guided by Dr. Conant's advice in calling this organism *Candida albicans*. At an informal meeting of medical

24. Zeisler, E. P.: Arch. Dermat. & Syph. **15**:171, 1927.

25. Engman, M. F., and Weiss, R. S.: Arch. Dermat. & Syph. **1**:119, 1920.

26. Frost, K.; Sutherland-Campbell, H., and Plunkett, O. A.: Arch. Dermat. & Syph. **20**:811, 1929.

27. Smith, E. C.: J. Trop. Med. **31**:101, 1928.

28. Clerf, L. H., and Bucher, C. J.: Ann. Otol., Rhin. & Laryng. **45**:923, 1936.

mycologists at the Third International Congress for Microbiology in September 1939, it was agreed to substitute the generic name of *Candida* for *Monilia*, pending official action by the rules committee of the International Botanical Congress (Martin and Jones <sup>23b</sup>).

#### SUMMARY

A case of *Candida albicans* infection is reported which during long periods of careful study in several clinics was considered to be a tuberculous infection. It is probable that this condition is not as rare as a study of the literature would indicate.

Mycosis should be considered in all cases of apparently nonspecific granuloma. In such cases Gram's stain is very useful in identifying the fungous elements in tissue sections.

A careful review of the literature reveals only 1 previously reported case of meningitis due to *Candida albicans*.



# General Reviews

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## THE BLOOD CHOLESTEROL

SIDNEY WEINHOUSE, PH.D.\*

CHICAGO

### I. NORMAL PHYSIOLOGIC VARIATIONS

Though cholesterol is found in virtually all tissues of vertebrates and invertebrates, its pathways of absorption, synthesis, destruction and excretion are unknown, as is also its part in the structure and the metabolism of living cells. It is not a foodstuff in the ordinary sense, yet its concentration in the circulating blood is of the same order as that of sugar and of fats. From a teleologic point of view, therefore, it is a substance of great physiologic significance. On the basis of its physical properties it is classified with the lipids, though chemically there is no relation. In chemical structure it resembles rather the terpenoid substance of plant origin. Indeed, the view was once held that cholesterol came exclusively from plants, and that the animal possessed no power to synthesize or to destroy it. Investigators know now, however, that it can be readily metabolized; and though nothing is known of its function in the organism, its chemical relatives, the bile acids and the adrenal and the androgenic and the estrogenic hormones, play a part in the most fundamental of the life processes.

The relationship between the blood cholesterol level and disease has been the subject of much clinical and experimental study, and an overwhelming amount of information has accumulated. Unfortunately, many conclusions which have been drawn rest on mistaken or confused ideas of what are normal cholesterol levels. The first part of this review will be devoted, therefore, to normal physiologic variations of the blood cholesterol. With this knowledge as a background, the metabolism of cholesterol and its relation to pathologic conditions will constitute the second and third parts of this review.

### METHODS OF DETERMINING CHOLESTEROL

Before discussing the blood cholesterol and its variations, a few words regarding its determination are necessary. Of the many color reactions given by sterols (Sobotka), the most popular is the Liebermann-Burchard reaction (Lieber-

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mann), utilizing the color developed by cholesterol in the presence of acetic anhydride and concentrated sulfuric acid. This reaction forms the basis of the quantitative methods of Grigaut (1910), Bloor (1916 a), Autentrieth and Funk and their innumerable modifications.

There are two serious objections to direct colorimetric estimation. First, an appreciable difference exists between free and bound cholesterol in the amount and velocity of the color development.<sup>1</sup> Second, the studies of Gardner and Williams, Yasuda, Reinhold, and Kelsey have shown that cholesterol esters yield values 10 to 30 per cent higher than the equivalent amounts of free cholesterol. Another source of error in the direct colorimetric procedure is the presence of interfering colors in the lipid extract and the possibility that other substances besides cholesterol in blood may contribute to the color (Reinhold).

For the precise determination of cholesterol, a method based on precipitation with digitonin is requisite. This essentially involves extraction of cholesterol and its esters by means of an organic solvent or mixture of solvents and precipitation of the free cholesterol before and after saponification by means of digitonin. The cholesterol content of the digitonide thus formed may be determined gravimetrically,<sup>2</sup> oxidimetrically<sup>3</sup> or colorimetrically.<sup>4</sup> The success of the gravimetric and oxidimetric methods rests on the assumption that the precipitated digitonide has a constant composition and that substances other than sterols are not precipitated. The first assumption is correct only if the factor is determined on each batch of digitonin used, as Schoenheimer and Dam found that the composition of the precipitate varies with the source of the digitonin. The second assumption requires qualification. Inasmuch as digitonin precipitates saturated sterols as well as cholesterol, the total sterol content rather than the latter is determined in these methods. Saturated sterols comprise only 1 to 5 per cent of the total sterols of body tissues (Schoenheimer, von Behring and Hummel); for all practical purposes, therefore, the total sterol and cholesterol may be considered identical. Other substances, particularly some of the steroid hormones, also are precipitated by digitonin. Though their concentrations in blood are negligible in comparison with that of cholesterol, they may amount to an appreciable fraction of the unsaponifiable matter of certain organs.

The recently reported method of Schoenheimer and Sperry has deservedly achieved popularity. In this procedure the digitonide after precipitation and subsequent removal of interfering substances is redissolved in acetic acid and the cholesterol determined colorimetrically by means of the Liebermann-Burchard reaction. Though originally adapted for the Step photometer, the color may be read conveniently by means of the photoelectric colorimeter. As digitonin does not produce an interfering color under the conditions of the method, the objections to the gravimetric and oxidimetric procedures are avoided, and as the cholesterol is free from other possible chromogenic substances and is determined only in the free state, the objections to the direct colorimetric procedure do not apply.

Another source of error in determinations of cholesterol is in the method used for its extraction. Those methods which involve preliminary drying of the blood or tissue by heating or by mixture with anhydrous chemicals, such as plaster of paris or sodium sulfate, are unsatisfactory (Gardner and Fox, 1924). Hot alcohol

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1. Mueller. Page and Rudy.

2. Ewert, 1933. Man and Peters, 1933. von Szent Györgyi. Windaus.

3. Boyd, 1933 a. Kirk, Page and Van Slyke. Okey, 1928.

4. Kelsey. Schoenheimer and Sperry.

apparently is the best extracting agent for cholesterol and other lipids, and its use is the basis of the method developed by Bloor (1928 d) for blood and tissue lipids. It has the virtues of completeness of extraction, avoidance of oxidation and simplicity. Man and Gildea (1932) reported that the method as described by Bloor, with a mixture of 3 parts of alcohol to 1 of ether being used, gave blood lipid values 5 to 31 per cent too low. Boyd (1936 d) attributed this to the use of insufficient extracting fluid. He found extraction was complete in five minutes if 25 parts of fluid to 1 part of blood were used.

In interpreting data on blood cholesterol, the matter of whether the determinations have been carried out on serum, plasma or whole blood should be considered. As the red cells contain only small amounts, if any, of cholesterol ester, and the total cholesterol content of the cells is generally lower than that of the plasma, values obtained for the whole blood will be characterized by a lower total cholesterol and an increased proportion of free cholesterol. Shope (1928) observed lower cholesterol ester values for oxalated or citrated plasma than for serum. He attributed this to partial hydrolysis of the cholesterol esters in the presence of the anticoagulant. This later more correctly was attributed to alteration of the plasma and red cell volumes brought about by the anticoagulant.<sup>5</sup> The resultant reduction in the concentration of the plasma may amount to as much as 15 per cent. Serum and heparinized plasma give identical values for free and total cholesterol, which more truly represent their concentrations in the noncellular portion of the blood (Sperry, 1937 i).

#### REPRESENTATIVE VALUES FOR BLOOD CHOLESTEROL

An overwhelming amount of clinical and experimental data regarding blood cholesterol has accumulated, and comparison is difficult when so many and such various methods of extraction and analysis have been employed. Of the thousands of reports on the blood cholesterol and its variations only a small proportion can be cited: those which in the author's opinion are of greatest significance.

Representative values for the concentration of cholesterol in the serum or the plasma of normal persons are those of Gardner and Gainsborough (1927 c) (169 and 153 mg. per hundred cubic centimeters of plasma for normal men and women, respectively, with standard deviations, respectively, of 41 and 33 mg. per hundred cubic centimeters); Okey and Boyden (149 mg. per hundred cubic centimeters, with a variation of 41 mg. for normal women); Man and Peters (1933) (207 mg. per hundred cubic centimeters, with a range from 162 to 258 mg.), and Boyd (1933 a) (162 mg. per hundred cubic centimeters, with a standard deviation of 20 mg., for normal women).

More recent studies have established higher normal average values as well as a wider range of normal variation. Kirk, Page, Lewis, Thompson and Van Slyke, in 1935, reported a study of the plasma lipids of 66 normal men ranging from 21 to 91 years. The values for

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5. Gardner, Gainsborough and Murray, 1938 c. Paget and Pierrart. Sperry, 1937 i.

the concentration of total cholesterol in plasma varied from 109 to 376 mg. per hundred cubic centimeters, with a mean of 232 mg. and a standard deviation of 62 mg. Age caused no significant variation. The values were higher than those obtained previously but were confirmed in another comprehensive study by Sperry (1936 f), who with 91 normal persons of both sexes found values ranging from 131 to 392 mg. per hundred cubic centimeters of serum, with an average of 209 mg. Some increase was noted with age. These results disclose that the range of cholesterol concentration considered normal in former years must be extended. In its variability cholesterol differs significantly from most of the other constituents of the blood, which normally fluctuate within narrow limits.

*Cholesterol Esters.*—Cholesterol, because of its alcohol group, can form esters, and in the blood a large proportion of it is thus combined with fatty acids. Constancy in the relation between the free and the esterified cholesterol of the blood was noted first by Bürger (1928), who reported that 30 per cent of the total blood cholesterol exists in the free form. Bloor and Knudsen reported a higher value: 40 per cent. Gardner and Gainsborough (1930 a, 1927 d), however, were unable to confirm this observation, nor could Kirk and his co-workers (1935), who reported values for the amount of free in the total cholesterol ranging from 22 to 72 per cent. In contrast to Kirk's results, the equally careful and comprehensive study of Sperry (1936 f) presented an entirely different picture. Though both investigators reported approximately equal ranges for the total cholesterol, Sperry found the ratio of free to total cholesterol to be remarkably constant. The amount of free in the total cholesterol varied between the narrow limits of 24.7 and 30.1 per cent, with a standard deviation of only 1.4 per cent from the average of 26.9 per cent. The reason for these differences is not evident. The ranges for the total cholesterol being approximately equal, they could not be attributed to inadequate extraction or to the use of serum in the one study and heparinized plasma in the other, for both have been shown to yield similar values (Sperry, 1937 i).

Further evidence for the constancy of the relation between free and total cholesterol in serum has been reported by Sperry in a large group of children (Sperry, 1936 g) and in over 100 persons who died by violence and whose blood was drawn post mortem (Landé and Sperry). Several others have since confirmed the constancy of this ratio in a variety of conditions,<sup>6</sup> and Gardner has modified his procedure and reported more constant values for the ratio (Gardner, Gainsborough and Murray, 1938 b).

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6. Muhlbock and Kaufmann. Offenkrantz. Offenkrantz and Karshan. Smith and Marble.

*Enzymes for the Synthesis and the Hydrolysis of Cholesterol Esters.*

—The remarkable constancy of the ratio between free and combined cholesterol in spite of variation in the total blood cholesterol indicates the existence of an enzyme system regulating their proportions in the blood. This subject only recently has been investigated intensively, with contradictory and confusing reports. Sperry (1936 e) found that esterification of free cholesterol occurred in serum incubated alone or diluted with saline solution. Esterification also proceeded when the serum was incubated with saline extracts of various tissues, though not to the extent with serum alone. Further studies (Sperry and Stoyanoff, 1937 c, d) led to the finding that the esterification occurring on incubation of dog and human serum was inhibited by bile salts. The inhibition was proportional to the concentration of the bile salts until a concentration was reached at which no esterification or hydrolysis occurred. With larger amounts than this, the proportion of free to ester cholesterol in human serum remained unchanged, but in dog serum an increase in the amount of bile salts caused complete splitting of the cholesterol esters. The greatest effect was obtained with the conjugated bile salts: taurocholate and glycocholate. Monkey serum acted like human rather than dog serum.

The enzymatic nature of this reaction was shown by the fact that no change occurred in serum heated for one hour at 55 C. (Sperry and Stoyanoff, 1938). When human or dog serum thus inactivated was incubated with unheated serum of either species, esterification occurred. In the presence of taurocholate dog serum and globulin fractions from dog serum promoted the hydrolysis of cholesterol esters in heat-inactivated human or dog serum. Human serum, on the other hand, or its globulin fraction did not promote hydrolysis of cholesterol esters in heat-inactivated serum of either species. Sperry concluded, therefore, that the esterification and the hydrolysis are independent, being catalyzed by different enzymes, both present in dog serum, but only one, the esterifying, in human serum. Riegel, Ravdin and Rose, however, in similar experiments, reported that hydrolysis occurs in both human and dog serum when these are incubated with bile salts.

## EFFECT OF AGE ON VALUES

At birth the blood cholesterol is distinctly low. In regard to infants from 4 to 25 days old, Sperry (1936 g) found values for heparinized plasma that ranged from 71 to 192 mg., with an average of 133 mg., per hundred cubic centimeters; after a pronounced increase during the first four days of life, the cholesterol level remained unchanged. Constancy of the ratio of free to total cholesterol, observed in adults, was not characteristic of infants' plasma, in which the amount of com-

bined cholesterol in the total varied from 41 to 72 per cent. Muhlbock and Kaufmann obtained values in close agreement with those of Sperry. Blood serum obtained from the umbilical cord at birth contained 70 mg. of cholesterol per hundred cubic centimeters, of which about 70 per cent was in the ester form. From the first to the fourth days of post-natal life, the average increased from 91 to 137 mg.; the values then remained constant through the twelve day period of study.

There is a gradual and slight increase with age throughout childhood,<sup>7</sup> the average values for boys and girls being about the same.<sup>8</sup> Basal metabolic rates and intelligence quotients could not be correlated with blood cholesterol levels,<sup>9</sup> and no differences have been observed between white and Negro children (Molitch and Poliakoff). The ratio of combined to free cholesterol was fairly constant in normal children, aged 2 months to 12 years, at  $2.67 \pm 0.55$ —about the same as the adult value (Offenkrantz and Karshan).

In a study of normal men, 21 to 91 years of age, Kirk and co-workers (1935) found no significant effect of age on the levels of cholesterol and its esters or other lipids in the blood plasma, nor any change in their proportions. Muhlbock and Kaufmann, on the other hand, observed an increase in free and in combined cholesterol with age in women. The variations were from an average of 200 mg. during the third decade of life to 260 mg. during the seventh decade. Several authors claim to have found lowered cholesterol values for normal persons in extreme old age.<sup>10</sup> Such conclusions are open to question, however, because of the possibility of nutritional or other metabolic defects in these subjects.

#### EFFECT OF PERSONAL AND ENVIRONMENTAL FACTORS

The wide normal range of the concentration of cholesterol in the blood raises the question of whether this variation represents differences between persons or variations in a single person over a period of time. The consensus of reports is that the cholesterol content of the blood of an adult is constant and independent of internal and external conditions. The cholesterol content of the blood of normal women during the intermenstrual period was found by Okey and co-workers (1927, 1933) to be constant, this observation being confirmed by Muhlbock and Kaufmann (1928, 1938) and Offenkrantz. The studies of Sperry (1937 i) over periods up to twenty-eight months on 25 adults of both sexes, and of Turner and Steiner over periods of seven to

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7. Baylac and Sendrail. Ward.

8. Rothbart. Ward.

9. Molitch and Poliakoff. Rothbart.

10. Brodin and others. Bürger and Möbius.

fourteen months on patients suffering from a variety of ailments, agree that the level of cholesterol remains remarkably constant for each person. The same conclusion was reached by Bloor (1933) on the basis of a study on dogs and by Boyd (1938) and Harnes (1928) in a study on rabbits. Man and Gildea (1937), on the other hand, after a study of 4 males and 6 females during periods of from three months to two years, and Schube after a study of 10 persons over a period of sixteen weeks, reported variations in the blood cholesterol level of single persons. Inspection of their data reveals that in the subjects of the former study the widest range of variance calculated as deviation from the average was only 15 per cent; in the latter, it was only slightly higher. From the widest fluctuations reported it is evident that the cholesterol level of the blood of a single person of a group is far more constant than the range of variation found in the group.

An interesting relation between the serum lipid levels and the body build has been shown by Gildea, Kahn and Man. They determined the serum lipids in men and women belonging to two distinct body types: the stocky, or heavy, pyknic, and the slender, asthenic. These subjects were normal and free from obvious endocrine disorders. They ranged in age from 18 to 50 years. The pyknic men had an average blood cholesterol concentration of 230 mg. per hundred cubic centimeters; the asthenic averaged only 168 mg. The slight difference found between the pyknic and the asthenic women, the averages being, respectively, 205 and 196 mg. per hundred cubic centimeters, was attributed in part to the difficulty in distinguishing the morphologic type in this sex. Offenkrantz confirmed these observations in unpublished data.

This relation affords an interesting field for speculation regarding the possibility of a connection between body build and susceptibility to diseases associated with abnormal deposition of cholesterol. Such speculation is useless, however, in the present state of ignorance of cholesterol metabolism, except possibly as an exercise of the imagination.

The effect of racial and climatic factors on the blood cholesterol has not been investigated extensively, but there is little variation between races living under widely differing nutritional and climatic conditions. With regard to subjects living in the Netherlands Indies, Radsma reported that 16 Europeans had an average blood cholesterol value of 191 mg., with a range of 130 to 290 mg., per hundred cubic centimeters, 33 native teachers and students 206 mg. and 45 servants and coolies 163 mg., the ranges approximating that of the Europeans. Gross found the blood cholesterol of East Indian natives about 40 mg. per hundred cubic centimeters less than that of Europeans or Americans living in the same region. Stone, after his study of South Rhodesian Negroes, suggested that these differences may be due to dietary factors, having

traced low cholesterol levels in these subjects to a low intake. Negroes living in the United States do not differ from whites in concentration of blood cholesterol. Blood cholesterol levels of Koreans are not significantly different from those of other races (Kim). These scattered data obviously need amplification before conclusions may be drawn as to the effects of heredity and dietary habits on the level of the blood cholesterol. Determinations on Eskimos, who live under dietary and other conditions differing widely from other races, reveal that such factors probably have little influence on the cholesterol levels (Corcoran and Rabinowitch).

Other environmental factors are temperature, pressure and light. Experimental work on dogs (Rabbeno) and rabbits<sup>11</sup> indicates that a decrease in atmospheric pressure causes a rise in free and total cholesterol, but the results of Müller and Talbott on human beings are not confirmatory. They found no change in the cholesterol or other lipids of the blood of 4 healthy young men raised from sea level to an altitude of 14,000 feet. Daily determinations made at the high altitude for thirty-nine days gave constant values. In a recent investigation by McLachlan on dogs and cats, reduction of the atmospheric pressure to less than one-half the normal value led to no change in any of the lipid fractions, whether the animals were in the fasting or the absorptive state. In rabbits the picture differed. After three hours at the low pressure there occurred a substantial decrease in neutral fat, a less pronounced decrease in phospholipid and no change in cholesterol. After six hours at the low pressure the values were at a normal level.

Well controlled experiments on the effect of external temperatures are lacking, but several authors<sup>12</sup> reported that no seasonal variation in the blood cholesterol was found. The effect of increases in the body temperature on the cholesterol level will be discussed later in connection with fevers and infections.

The effect of electromagnetic radiation of various wavelengths has been studied, but the results are contradictory. Malczynski<sup>13</sup> reported that radiation from a quartz lamp produces a temporary increase in blood cholesterol. A similar rise was observed on irradiation with roentgen or infraroentgen, solar and even infra-red rays. Kasatkin and Bugdanova observed this transient increase, but Ornstein reported that the variations in the blood cholesterol of 25 patients treated with ultra-violet rays were not significant. The studies of Harnes (1929) on the effect of various light environments on the blood cholesterol of

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11. Griffel. Starup. Wischnowitzer.

12. Boyd, 1938. Man and Gildea, 1937.

13. Malczynski. Malczynski and Lankosz.



rabbits did not establish any significant differences between animals kept in total darkness and those subjected to visible or ultraviolet radiation.

A factor which probably has not received the attention it deserves is the emotional state of the subject at the time of bleeding. Lyons observed that cats within twenty to forty minutes after a period of excitation had hypercholesteremia, the cholesterol being 25 to 30 per cent above the original level. Cholecystectomy did not influence the observed increase, thus ruling out the possibility of a sudden contraction of the gallbladder, followed by resorption of the cholesterol, but the effect was abolished after sympathectomy. Additional evidence for the effect of the mental state on the blood cholesterol has been obtained by Dobreff, Peneff and Wittkower. Of 9 patients from whom blood was drawn immediately before an operation, 6 had a significant increase of blood cholesterol, 1 a slight increase and 2 a slight decrease. Of 16 subjects whose blood was drawn during hypnosis, 11 had a slight to great decrease, whereas 5 had the same or only slightly decreased values.

The effect of muscular work on the blood lipid levels has been investigated by Fahrig and Wacker. After fifteen to eighty minutes of extreme muscular effort increased values were observed for all the lipid fractions, including free and total cholesterol; the data, however, are meager.

#### CHANGES DURING MENSTRUATION AND PREGNANCY

Exceptions to the usual constancy of the blood cholesterol are the changes in women during menstruation and pregnancy. From data obtained from determinations of the lipid content covering twenty-six monthly cycles in 16 normal young women, Okey and Boyden showed that definite changes in the blood cholesterol levels occurred. The usual fluctuations during the menstrual cycle involved a slight premenstrual rise, followed by a distinct fall immediately before or during menstruation, then a sudden rise during or slightly after the bleeding phase, followed by a gradual decline to the average value. These changes occurred over a period of two weeks and usually were not paralleled by similar fluctuations in the fatty acid and "lecithin" levels. Kaufmann and Erdmann also observed a fall in blood cholesterol during menstruation, which involved principally that in the free form. During the intermenstrual period, about 70 per cent of the total cholesterol was in the form of the ester, whereas during menstruation 85 to 90 per cent of the cholesterol was esterified. These regular variations were considered to be related to ovarian function, as they were abolished by ovariectomy or the physiologic climacteric. In a later publi-

cation Muhlbock and Kaufmann confirmed the intramenstrual fall in the blood cholesterol but reported that the decrease was confined to the esterified cholesterol. Offenkrantz also found a decrease in the total serum cholesterol at the onset of menses, and a rise at the end of the bleeding phase. The changes were mainly in the ester cholesterol fraction and were slight.

That the cholesterol content of the blood increases during pregnancy was pointed out many years ago by Grigaut (1913) and Hermann and Neumann and has been confirmed by many reports which have been summarized adequately by Boyd (1934) in his excellent review on lipemia of pregnancy. From the consensus of studies on this subject, the lipid picture in the blood of pregnant women may be described as follows: During the first trimester there is no change. A gradual rise in all the lipid constituents begins with the fourth month and continues up to the eighth month. The levels thereafter remain high through the puerperium, then decline gradually to normal. Tyler and Underhill observed increases in the levels of free and combined cholesterol and phospholipid, beginning at the third month and continuing to term, after which the levels remained high about two weeks. The ratio of free to total cholesterol and that of phospholipid to cholesterol were fairly constant throughout the period. Boyd (1934) has shown that the lipemia of pregnancy involves changes only in the plasma, the erythrocyte lipids remaining constant. The greatest changes were in the neutral fat fraction, which began to rise in the first trimester and at term was about 100 per cent above the level observed in the absence of pregnancy. The phospholipid and the free and the combined cholesterol began to rise in the second trimester and at term were about 25 per cent higher on the average than in nonpregnant women, though the relative proportions of these substances were unaltered. Similar findings have been reported by Muhlbock and Kaufmann regarding the constancy of the ratio of free to total cholesterol during pregnancy.

The recent publication of Schwartz and associates is of especial interest in that the changes in the blood lipids of the same subjects were followed from the beginning to the end of pregnancy. In general, the changes resembled those in previous studies, namely, moderate increases in free and esterified cholesterol, phospholipid and glyceride, with maintenance of a fairly constant composition of the lipid fraction.

The lipemia of pregnancy, established beyond question in the human species, has not been observed invariably in animals. No variations were noted in the blood lipids of rats (Kaufmann and Erdmann) and dogs (Baumann and Holly, 1926) during pregnancy, and in herbivora there appears to be an actual decrease in the blood lipids during this period. Baumann and Holly (1926) observed in pregnant rabbits

decreases in the cholesterol and the phospholipid to about one-half the values for nonpregnant ones. The decrease occurred during pregnancy even in thyroidectomized rabbits, whereas normally thyroidectomy raises the lipid levels. Boyd (1936 h) confirmed these results and found decreases in this species even in pseudopregnancy. In cows the "dry" period of pregnancy was characterized by low lipid levels in comparison with the period of lactation.<sup>14</sup> Several reports, however, indicate that blood cholesterol may be raised during pregnancy in cows (Sato), rabbits (Kawaguchi) and horses.<sup>15</sup>

Many attempts to explain the lipemia of pregnancy have been reviewed by Boyd (1934), who himself suggested that the increased lipids may act as a "pressure head," forcing the lipids from the maternal blood through the placenta into the fetal circulation. Boyd and Wilson showed that large quantities of lipids pass through the placenta and are removed by the fetus. The amount of lipid absorbed to satisfy the nutritional requirements of the growing fetus was estimated to be of the order of 40 to 50 Gm. per day. The major portion, phospholipid, constituted about 75 per cent of the total. A small, though definite, amount of cholesterol likewise is removed. Although Boyd thus proved that cholesterol and other lipids are supplied to the child by the mother, it is doubtful if the lipemia plays a part, for lipemia does not occur in all species during pregnancy.

Boyd (1935 f) studied the variation in the blood lipids of women during the puerperium. He found that the plasma lipids began to decline almost immediately after the birth of the child, though the decrease was confined almost exclusively to the neutral fat fraction. The phospholipid and cholesterol values remained relatively constant. A significant difference existed between nursing and non-nursing mothers. If normal lactation ensued, the puerperal drop in lipids continued; if the breasts were "dried up," a secondary rise brought the level to or above that of pregnancy.

#### EFFECT OF DIET ON THE BLOOD CHOLESTEROL

*Effect of Hunger.*—The effect of fasting was studied first by Gardner and Lander, who observed in cats and rabbits considerable increases in free and esterified cholesterol during periods of hunger. These observations were confirmed by Shope (1927) in human beings, pigs, cats and guinea pigs. During periods of fasting up to six days, hypercholesteremia developed, which decreased a few hours after feeding. The decrease in the blood cholesterol occurred regardless of the type of food fed (fat, carbohydrate, protein or mixed). Similar findings

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14. Maynard and others. Shope and Gowen.

15. Brocq-Rousseau and others. Muhlbock.

in rabbits were reported by Fahrig and Wacker. Man and Gildea (1936), on the other hand, reported a study of 10 malnourished patients in whom the cholesterol content varied directly with the state of nutrition. Decreased cholesterol during inanition was reported many years earlier by Terroine, but examination of the protocols shows the changes to have been insignificant.

In regard to rats, Sure, Kik and Church found no changes in the blood cholesterol during fasts up to twenty-six days, though large decreases were observed in the fatty acids and phospholipid. A recent study indicates that animals may be starved to a considerable loss in weight without affecting the levels of the blood lipids. Entenmann, Changus, Gibbs and Chaikoff reported no significant changes in the concentrations of cholesterol, phospholipid and fatty acids in the blood of dogs fasting from four to thirty days or chronically undernourished for several months. In the acute fasting experiments, in which only salts and vitamin concentrates were given, all the lipids were maintained near the control levels up to thirty days. In chronic undernutrition extending up to five months and resulting in a loss in weight of 50 per cent, some lowering of lipid levels was found, being greatest for the fatty acids.

*Effect of Diet.*—The effect of diet on the concentration of cholesterol in the blood over extended periods was reported first by Luden, who by self observation found that a prolonged vegetable diet resulted in a decreased value for cholesterol and an exclusive meat diet in increased values. Gardner and Gainsborough (1927 c), also on the basis of observations of a single person, reported that the blood cholesterol level varied with the amount of cholesterol in the diet. Turner and Steiner observed 9 patients for twelve to fourteen months and concluded that no relation existed between the type of diet and the blood cholesterol values. Neither maintenance on a high or a low fat diet nor addition of cholesterol to the food influenced the blood cholesterol. Okey and Stewart determined cholesterol in the whole blood of 4 normal women who for approximately one month were kept on diets containing varying amounts of cholesterol. Values were slightly higher when the diet contained 3.1 Gm. of cholesterol per day than when a control diet was used, containing only 0.77 Gm. Values were higher when the cholesterol was administered in the form of egg yolks instead of pure cholesterol mixed with butter. The differences were slight, however. Corwin reported that long term feeding of a high fat diet produced only slight hypercholesteremia in dogs, but when the high fat diet was supplemented with lecithin there was marked elevation of free and combined cholesterol. Cholesterol administered in the solid state without fat had no effect on the blood levels.

The specific effect of lecithin on the level of the blood cholesterol in persons to whom it was fed was investigated by Steiner and Doman-ski. Ten patients were given 100 Gm. of egg yolk powder daily for six to ten weeks. The daily intake on this diet was 14 Gm. of lecithin and 8 Gm. of cholesterol. The serum cholesterol rose 40 to 218 mg. per hundred cubic centimeters, with an average increase of 101 mg. Similarly, in 4 dogs the same diet resulted in increases in the cholesterol ranging from 155 to 251 mg. per hundred cubic centimeters. Treadwell and Eckstein effectively demonstrated the lack of influence of fat feeding on the blood cholesterol levels in rats. The average values for free and combined serum cholesterol were the same for groups of animals fed diets containing 6 and 28 per cent fat.

Thus, ingested cholesterol, alone or mixed with fat, has little or no influence on the level of cholesterol in the blood, but when mixed with lecithin it produces appreciable rises in the level, probably through finer emulsification and more efficient absorption.

The interesting experiment reported by Tolstoi in which Stefansson, the Arctic explorer, and a friend, two apparently healthy men, subsisted for one year on meat alone is worthy of note. Their diet, in which fat comprised about 75 per cent of the total calories, contained an estimated 2 to 5 Gm. of cholesterol per day. In one the plasma cholesterol rose slightly, from an initial value of 263 to a maximum of 315 mg. per hundred cubic centimeters, in a few days. fluctuated between these values throughout the experiment and at the end was actually lower than at the start, 212 mg. In the other, unfortunately, the initial value was not obtained. During the course of the experiment the values ranged from 268 to 800 mg. per hundred cubic centimeters, the final value being 415 mg. After four weeks on a general (low cholesterol) diet, the value decreased to 200 mg. per hundred cubic centimeters. The results do not disclose any clearcut effect of a long term, high cholesterol diet on the plasma levels of this lipid. but they emphasize the factor of individual tolerance.

Reports regarding diurnal variations in the blood cholesterol disagree, but the weight of evidence indicates that the changes are not significant. Iscovesco claimed to have observed two to three maxima in the blood cholesterol throughout the course of a day, corresponding to periods six to eight hours after meals, but this has not been borne out by later investigations. Variations found during the course of a day by McEachern and Gilmour, and Fröhling could not be correlated with the type or the amount of food fed. Munoz, and Bruger and Somach reported the diurnal cholesterol level to be unaffected by the type of diet and found only slight variations. In determinations carried out every two hours for twenty-four hours on 9 subjects

(Bruger and Somach) the variations in the whole blood cholesterol averaged less than 8 per cent. Over a four hour period, either absorptive or fasting, the standard deviation was only 4 per cent. Results were similar in a careful study carried out by Boyd (1935 c). Eight adult subjects on a balanced diet of three meals per day were studied. Blood was drawn at intervals during the day and night. The cholesterol and other lipid values were not affected by such factors as time of day, intake of meals or sleep. The level remained quite constant. Daily variations expressed as standard deviations from the mean were 6.5 and 7.2 per cent for free and ester cholesterol, respectively—one-half to one-third the variation among different persons.

*Alimentary Hypercholesteremia.*—Early observations<sup>16</sup> indicated that an increase in the blood cholesterol occurs after ingestion of food which may contain little or no cholesterol. McClure and Huntsinger reported that the cholesterol levels rose after adults were fed meals of oleic acid or olive oil. Page, Pasternak and Burt, and Wendt, found increases in the blood cholesterol occurring within four hours when 100 Gm. of olive oil was fed to normal fasting subjects. Others have reported that ingestion of large quantities of fat had no influence on the blood cholesterol in normal and nephritic subjects (Hiller and associates), normal and xanthomatous patients (Chaikoff and co-workers), children (Wilson and Hanner) and dogs.<sup>17</sup> Blotner attempted to apply the changes in the cholesterol content of the blood occurring after ingestion of fat as a diagnostic criterion in various clinical conditions. The method is similar in principle to the sugar tolerance test. Blood is taken before, and at hourly intervals after, ingestion of 1 pint (453 cc.) of 20 per cent cream, equivalent to about 100 Gm. of fat. Normal persons show no significant changes in an eight hour period. In a group of thin persons the blood cholesterol remained constant or decreased slightly; in a group of 21 obese but otherwise apparently normal subjects the levels during fasting were higher than in a similar group of normal controls, and the cholesterol curve was characterized by a considerable increase at six hours, followed by a gradual decline. These results in obese persons contradict data reported by Rony and Levy, who found no definite change in the plasma cholesterol values of 18 markedly obese subjects after these had ingested 1 pint of 20 per cent cream.

The high cholesterol values sometimes found in cases of advanced or uncontrolled diabetes have stimulated investigation of the effect of dextrose on the cholesterol level. Remesow and Mattrosowitsch reported that cholesterol and dextrose varied inversely in the blood. Partly on this basis, they concluded that cholesterol may be converted

16. Autentrieth and Funk. Lifschutz. Lindemann.

17. Bloor, 1933. Rubin.

to carbohydrate. Mosenthal studied the effect of the ingestion of dextrose on the level of dextrose and that of cholesterol in the blood. He found that the rise in blood sugar was accompanied by wide variations in the cholesterol concentration, but no regular behavior was observed; the cholesterol increased, decreased or remained constant. The author concluded that osmotic phenomena may have played a part in the variations observed. Fitz and Bruger, in carrying out the usual dextrose tolerance test on 20 patients, observed an increase in the cholesterol ester fraction of the blood serum of 12. In the remaining 8, it was constant or decreased slightly. Sperry (1937 h), on the other hand, was unable to confirm these results, and concluded that no significant variation in the cholesterol occurred as a result of the ingestion of dextrose. The experimental work of Fitz and Bruger was criticized on the basis that their subjects were bedridden patients, many of whom exhibited cachexia and conditions such as infectious arthritis, carcinoma and hepatic cirrhosis.

Bruger and Poindexter reported that intake of large quantities of water had no effect on the level of the blood cholesterol; with water accompanied by varying quantities of urea, slight lowering of the cholesterol levels was observed, which probably was not significant.

*Hypercholesteremia Following Ingestion of Cholesterol.*—The notion that the blood cholesterol is increased after the ingestion of this lipid rests on the results of Bürger and Habs, who substantiated many similar earlier observations. They studied a group of fasting normal persons, who were given 100 cc. of olive oil containing 5 Gm. of cholesterol or cholesterol esters. In each instance lipemia occurred within four hours, accompanied by considerable increase in the free and the combined cholesterol. The levels declined thereafter and reached the normal fasting value within twelve hours. The negative results of previous investigators in similar experiments<sup>18</sup> were attributed to the administration of insufficient cholesterol, the use of inadequate analytic methods or the use of too little fat in admixture with the cholesterol. Gardner and Gainsborough (1927 c) were unable to confirm this and concluded that there is no relation between the amount of cholesterol ingested and the level of this lipid in the plasma. They criticized the results of Bürger and Habs because an inadequate method of extraction had been used. Bürger (1928) applied this procedure as a diagnostic test in a variety of conditions. Barreda, however, carried out the same procedure in normal and in abnormal subjects without observing the consistent changes reported by Bürger. Inasmuch as large quantities of cholesterol must be ingested to produce even questionable hypercholesteremia, this condition apparently does not occur when cholesterol is taken in physio-

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18. Campbell. Cohn and Heimann. Mjassnikow, 1926. Rothschild, 1915. Ssokoloff.

logic quantities, and under normal dietary conditions the fluctuations in the concentration of cholesterol in the blood appear to be of little importance.

*Effect of Cholesterol Feeding in Herbivora.*—Though in man and in other omnivora dietary cholesterol has little or no influence on the level of this lipid in the blood, in rabbits the reaction to the ingestion of this substance is marked. When cholesterol is added to the food of these animals to the extent of only a fraction of a per cent of the diet, the blood levels are raised and cholesterol is deposited extensively throughout the body. Since the deposit of lipids in the large arteries of rabbits fed cholesterol resembles human atherosclerosis, a voluminous literature has accumulated which has been reviewed by Schoenheimer, (1924) Duff and Anitschkow. The pioneering work of Wacker and Hueck established that the blood cholesterol of rabbits, normally low, hav-

*Results Obtained in Two Investigations of the Effect of Cholesterol Feeding in Rabbits*

Authors	Free Cholesterol, Mg. per 100 Ce.	Total Cholesterol, Mg. per 100 Ce.	Phospholipids, Mg. per 100 Ce.	Glycerides, Mg. per 100 Ce.	Total, Mg. per 100 Ce.
Page and Bernhard*...	356	1,435	526	270**	2,902
Weinhouse and Hirsch†	452	1,652	706	718	3,660

\* Cholesterol, 0.2 Gm. per day, was administered as a solution in oil. The determinations were made on heparinized plasma.

† Cholesterol, 1.0 Gm. per day, was administered without added fat. The determinations were made on serum.

\*\* This value was calculated from the authors' figures by subtracting the several lipid fractions from the total lipid.

ing average values of 20 and 50 mg. per hundred cubic centimeters for free and total cholesterol, respectively, increased after several months of cholesterol feeding to approximately twenty times these values. Versé confirmed these results but observed that the high cholesterol levels are reached much more quickly and with smaller daily feedings of cholesterol when it is administered as a solution in oil by means of a stomach tube. Cholesterol unquestionably is absorbed and rises to high levels in the blood without the addition of oil.<sup>19</sup> The only complete determinations of lipids in cholesterol-fed rabbits were carried out by Page and Bernhard and Weinhouse and Hirsch. The effect of added fat is well illustrated by the fact that in the two studies approximately equal values for free and total cholesterol were found though in the latter investigation five times as much cholesterol was administered as in the former, in which oil was used as a vehicle. The accompanying table

19. Bruger and Fitz. Meeker and others. Turner and Bidwell. Weinhouse and Hirsch.



shows the values reported for the separate lipid fractions at the height of the hypercholesteremia.

In spite of the difference in the administration of the cholesterol in the two studies, approximately the same relationships are noted between the free and the combined cholesterol, and phospholipids, though the low value for glycerides in the animals on the high fat diet is puzzling.

Weinhouse and Hirsch observed a gradual decline in the values for all the blood lipids after the maximum levels had been reached in about three months. They attributed this to impairment of the absorption of food as a result of infiltration of lipids into the transporting cells of the small intestine, which was observed histologically. The gradual loss in weight which paralleled the decline in lipid values substantiates this hypothesis.

No satisfactory reason has been found as to why rabbits differ from omnivora in their ability to dispose of dietary cholesterol. Schoenheimer pointed out that rabbits and guinea pigs differ from omnivora in that they do not have alimentary lipemia<sup>20</sup> after a single large feeding of fat. They are the only animals in which lipoidosis occurs as a result of cholesterol feeding. It seems reasonable to assume that the rabbit is reacting to a substance foreign to its diet by virtue of its herbivorous nature. But all animals, including rabbits, contain cholesterol as a normal component of their tissues and body fluids, and presumably possess mechanisms for its excretion, destruction and synthesis. It is difficult to understand, therefore, how the rabbit can distinguish between cholesterol of exogenous and cholesterol of endogenous origin. It undoubtedly lacks some excretory or metabolic function, possessed by omnivora, for the removal of exogenous cholesterol. At present, no anatomic or physiologic difference is known to account for this unusual behavior. With the exception of guinea pigs,<sup>21</sup> which react similarly to rabbits, there are no data on whether or not the susceptibility to lipoidosis following cholesterol feeding is shared by other herbivora.

#### PHYSICAL STATE OF CHOLESTEROL IN THE BLOOD

Under ordinary circumstances the blood plasma is clear and transparent in spite of the large amounts of cholesterol, glycerides and other lipids in it. As these substances are extremely insoluble in aqueous mediums, it is assumed that they are present in the blood in colloidal form. Studies of the colloidal properties of aqueous solutions of cholesterol and its esters, alone or combined with other lipids, have not been of great value in elucidating the state of these substances in the blood. The physical properties of cholesterol sols reported by various

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20. The term "lipemia" denotes the typical milky appearance of the serum determined by visual observation and has no chemical significance.

21. Rothschild, 1915. Wacker and Hueck.

investigators should be viewed with caution because in most instances the materials used were highly impure and because the properties in question often are affected profoundly by the method of preparation of the sols. Moyer, for example, observed considerable divergence in the isoelectric point and electrophoretic mobility at  $p_H$  5.8 of cholesterol sols prepared according to the directions of other investigators.<sup>22</sup>

In general, colloidal solutions of cholesterol and its esters are difficult to prepare, are precipitated easily by low concentrations of salts, even when stabilized by phospholipids,<sup>23</sup> and always have a milky appearance even in great dilution. According to Remesow (1936), sols of highly purified cholesterol cannot be prepared, for they coagulate at the instant of formation even with the lipid in extremely low concentration.

The relative stability of the plasma lipids as compared with their colloidal solutions in vitro suggests a combination with the plasma proteins; indeed, most investigators agree that the lipids are "peptized" in this manner. As evidence of such a combination it has been repeatedly demonstrated that only a fraction of the lipids can be extracted from plasma by the usual lipid solvents and that they tend to remain with the protein fractions precipitated by salts (Turner and Gibson).

When serum is treated with solvents, such as ether or chloroform, the lipids are removed only incompletely.<sup>24</sup> The amount that can be extracted varies with the  $p_H$ , being greatest at 5.5 to 6.0, the isoelectric point of globulin and fibrinogen.<sup>25</sup> Over the  $p_H$  range of 1.7 to 13.3, only a small fraction of the total is extracted with ether (Delage, 1936 a). If a small quantity of lower alcohol or acetone is added to the serum, the greater part of the lipid is extractable by ether (Delage, 1936 b). This is attributed to a decrease of the interfacial tension between the serum and the ether, resulting in more intimate contact between the latter and the protein-lipid micelles; but it may likewise be attributed to denaturation of the protein.

The precipitation of cholesterol with the salting out of proteins has been observed by many. The early literature was reviewed by Turner and Gibson. The amounts of the various lipids precipitated with the protein fractions differ according to variations in the experimental procedures employed, with differences in findings between many investigators; but all agree that relatively large proportions are combined with the protein of the serum.

Turner and Gibson fractionated the proteins of horse serum and human and dog plasma by salt precipitation and determined fatty acids, phospholipids and cholesterol in these fractions. About half the total

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22. Eagle. Kermack and MacCallum. Keeser. Porges and Neubauer. Rona and Deutsch. Stern.

23. Remesow, 1936. Stern.

24. Achard. Delage, 1936 a. Handovsky and others. Neuschloss. Terroine.

25. Delage, 1936 a. Theorell.

lipids were carried down with the proteins. Greater amounts were associated with the globulin than with the albumin. For all species, about 70 per cent of the free and of the total cholesterol was carried down with the proteins and was equally distributed between the albumin and the globulin.

By successive fractionation of horse serum with ammonium sulfate at  $p_H$  3.8, Macheboeuf isolated an albumin fraction containing 22 per cent "lecithin," 18 per cent cholesterol esters and 60 per cent protein. In spite of its high lipid content, this substance was very soluble in water. Clear solutions containing more than 50 Gm. of lipids per liter and clear gels containing more than 100 Gm. per liter were obtained. The lipids could not be extracted directly from these solutions by ether but were extractable after the protein had been coagulated with boiling alcohol. The isolated lipids were not soluble in water.

Kleczkowski showed that the amount of lipid as well as carbohydrate combined with the serum albumin depends on the method of the latter's preparation. Crystalline serum albumin of the horse, for example, contained only traces of cholesterol, phospholipid and sugar, whereas the albumin of high lipid content prepared according to Macheboeuf contained as much as 1 per cent of sugar. Albumins prepared in other ways contained varying quantities of lipids and carbohydrate.

Ultrafiltration studies of serum and other body fluids also indicate that proteins are combined with cholesterol. Went and Goreczky showed that the cholesterol concentration in serum ultrafiltrates was proportional to the protein concentration. When the ultrafiltrate was protein free, it was also cholesterol free. The phospholipid, on the other hand, seemed associated predominantly with the euglobulin fraction, since only a small proportion passed through with the protein of low molecular weight.

In similar experiments, Bendien and Snapper reported that neither lecithin nor cholesterol was combined with the albumin but may have been partially associated with the euglobulin. In a study of pathologic pleural and ascitic fluids, Bruger found that though the cholesterol did not pass an ultrafilter permeable to protein it was readily adsorbed on kieselguhr. Bruger expressed the belief that this indicates that cholesterol is not combined with protein, but this argument is not valid. It is probable that the cholesterol, united by loose chemical forces to the protein, has an affinity for kieselguhr great enough to overcome the hold of the protein.

Application to this problem of the improved electrophoresis apparatus recently developed by Tiselius has furnished the most convincing evidence for the presence, in normal serum, of combinations between the proteins and cholesterol. Tiselius showed that serum contained four well defined protein components which migrated at different velocities

in the electrophoresis apparatus; albumin and three globulins, denoted  $\alpha$ ,  $\beta$  and  $\lambda$ , respectively. By analyzing these fractions, Blix, Tiselius and Svensson demonstrated that all contained cholesterol, with far greater quantities in the  $\alpha$  and  $\beta$  globulins than in the other two components of the serum. These fractions were richer also in phospholipid and carbohydrate. Mellander had shown previously by electrophoresis that the albumin and globulin of serum contained cholesterol; also, that a negatively charged cholesterol fraction migrated with a greater velocity than the proteins. Thus, at least a part of the cholesterol is unbound. He also noted cholesterol-free albumin and globulin fractions.

Though protein-lipid combination undoubtedly occurs, the character of the forces involved is not clear. It is doubtful that the lipids are attached as a prosthetic group by the ordinary chemical bonds, for they are removed by comparatively mild procedures not involving the integrity of the protein except possibly for denaturation. Two other possibilities suggest themselves. Combination may occur through the mutual attraction of oppositely charged polar groups, resulting in a uniform distribution of discrete lipid molecules throughout the protein micelle. Or, combination may take place between the protein micelles and high molecular aggregates of lipid molecules, held together by opposite electrical charges or by physical enmeshing as suggested by Bruger (1935 a).

The significance of these protein-cholesterol combinations to the organism other than as a possible means of holding the cholesterol in solution is not established. Wide variations may occur in either component without affecting the other. For example, Schwarz and Lichtenberg found that the hypercholesteremia induced by bleeding had no effect on the serum protein levels, and Page, Farr and Weech observed no consistent changes in the total concentration or the relative proportions of the serum lipids in dogs fed a low protein diet until the serum albumin dropped below 1.5 Gm. per hundred cubic centimeters and gross edema occurred.

Evidence indicates that in contrast to its relative nonreactivity in the molecular state, colloiddally dispersed cholesterol is markedly chemically active. For example, Remesow and Mattrosowitsch found that cholesterol sols have a strong reducing action toward the usual sugar reagents. Colloiddally dispersed cholesterol and its esters in the presence of air or of hydrogen peroxide can dehydrogenate polyamines and phenols (Remesow and Sepalowa, 1933, 1935) and under certain conditions may have a catalase activity (Remesow, 1934). These findings suggest the possibility that cholesterol may take part in cellular respiration. Further work along these lines is obviously necessary.

## THE CHOLESTEROL OF THE RED CELLS

The reported values for the concentrations of cholesterol in the red blood corpuscles of normal human beings range from 62 to 240 mg. per hundred grams of cells. Grigaut and L'Huiller reported values from 130 to 177 mg.; Bloor (1916 c), 170 to 240 mg.; Oser and Karr, 62 to 156 mg.; Laroche and Grigaut, 150 to 165 mg.; Brun, 127 to 149 mg., and Boyd (1936 g),  $150 \pm 19$  mg. These data show that though the average values differ considerably, probably reflecting differences in analytic procedures, the range of variation reported by each author is narrow compared with the normal range of variation observed in the serum.

In contrast with that of the serum, the cholesterol of the erythrocytes is mainly, if not entirely, in the free form, though investigators disagree as to the actual amount of combined cholesterol present. Richter-Quittner, Iwatsuro, Brun, Sperry (1935) and Rubin were unable to find statistically significant quantities of combined cholesterol in the cells of human beings, but others<sup>26</sup> reported slight, though definite, amounts. In animals as well, some investigators<sup>27</sup> found minimal quantities of ester cholesterol or none, whereas others observed appreciable amounts.<sup>28</sup> How much of this disagreement is due to errors in the analytic methods is impossible to estimate at present. Aside from the usual sources of error encountered in determinations of cholesterol, one applies particularly to the red cells. When the cellular concentration of a substance is calculated indirectly from the difference between whole blood and plasma values on the basis of the hematocrit reading, the error is large, because the concentration must be calculated from three experimentally determined values, each of which is subject to some error. This criticism applies especially to combined cholesterol, the concentration of which in the cells is low and which itself is not determined directly but is calculated from the difference between the total and the free cholesterol. Consequently, the direct method, in which a measured portion of the cells is analyzed after preliminary separation of the plasma, is preferred. After comparing the direct and the indirect method for the determination of corpuscular lipids, Boyd (1936 g) concluded that only the former yields reliable figures.

The cholesterol content of the erythrocytes undoubtedly remains constant under conditions in which there is considerable variation in that of the plasma. Boyd (1934 e) has shown that the cellular lipids are unchanged in the chronic lipemia of pregnancy, of diabetes and of hemorrhage. These observations were confirmed strikingly by Rubin,

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26. Boyd, 1936 g. Cytronberg. Erickson and others. Rosenthal.

27. Bodansky, 1925. Knudson. Wacker and Hueck.

28. Barreda. Pfeiffer. Rohmann.

who found normal values for cholesterol and the other lipids of the red cells in a diabetic patient whose plasma showed 1,600 mg. of cholesterol and 23,000 mg. of total lipids per hundred cubic centimeters. In alimentary as well as in chronic lipemia no change is noted in the cholesterol of the red cells. Bloor (1916 b) and Rubin with dogs, and Iwatsuro, with rabbits, observed no increase in the concentration of cholesterol in the red corpuscles after feedings of fat with or without cholesterol. Similar observations were made by Wendt, Fröhling and Boyd and Twedell in human subjects. Slight increases in the cellular cholesterol after cholesterol feeding were reported by Knudson and Bodansky (1931) in dogs, and by Henes, Richter-Quittner and Brun in human beings. Brun found no variations in the cholesterol of the red cells in human beings over periods of one day or a month. He observed no effect of age.

Though the variations in plasma cholesterol are not reflected in the erythrocytes, there are some indications that the latter undergo changes in their cholesterol concentration under certain conditions. Boyd (1936 g) observed increases in the red cell lipids not related to similar changes in the plasma lipids in cases of infection and of anesthesia induced with ether. Bloor (1916 c) observed increased cholesterol in the red cells of only a few of a large group of subjects suffering from various pathologic conditions; in the majority the cholesterol content was within normal limits. Brun reported slightly higher than normal values for patients suffering from cancer and markedly higher values for those suffering from jaundice, the values decreasing as the conditions improved. The increases in the jaundiced patients were attributed to the enhanced absorption of cholesterol under the influence of the bile acids, which were at high levels in the blood in this condition.

Bugnard observed that the venous plasma of dogs contained more cholesterol than the arterial plasma, whereas the concentration in the whole blood was unchanged. He concluded that there was an exchange of cholesterol between the corpuscles and the plasma depending on the  $p_H$  of the circulating blood. According to his theory, when carbon dioxide is given up by the blood in the lungs, and the blood becomes relatively alkaline, cholesterol migrates from the plasma to the corpuscles. Then, as the blood again is saturated with carbon dioxide during its passage through the tissues, the reverse change takes place. In confirmation, Bugnard found in experiments in vitro that saturation of whole arterial blood with oxygen gave a plasma poorer in cholesterol than when the blood was saturated with carbon dioxide. Similarly, acidification of whole blood with ammonium acid phosphate produced a distinct rise in the plasma cholesterol. Levine and Soskin also found an inverse relation between the total serum "fat" and its carbon dioxide-combining power. The "fat" content of the whole blood remaining

unchanged, they postulated a labile exchange of "fat" between the corpuscles and the serum under the influence of the acid-base balance.

If such a process occurred under the influence of the  $p_H$ , the oxygenated serum leaving the lungs should contain less cholesterol than the venous blood serum of the right side of the heart. Shillito, Bidwell and Turner determined the cholesterol content of whole blood and serum taken simultaneously from the superior vena cava and the carotid artery. For a given animal (dogs and cats were used) the levels were the same. Thus no effect of passage through the lungs on the relative proportions of cholesterol in the red cells and the serum could be demonstrated.

Studies have shown that the lipids of the erythrocyte are not distributed uniformly throughout the cell, but are associated with the stroma.<sup>29</sup> Indeed, they are bound so firmly as to withstand repeated washing with buffer solution. Analyses of stroma from several species of animals show varying absolute quantities of lipids, but the relative proportions remain fairly constant at about 60 per cent phospholipid, 30 per cent free cholesterol and 10 per cent cholesterol esters and neutral fat (Erickson and co-workers). Essentially similar values have been reported for the erythrocytic lipids of a large variety of mammals and fish (Dzemian). The amounts of cholesterol and other lipids appear related to the size of the cell; the larger the cell, the greater the percentages.

The chemical and physical properties of the erythrocyte suggest that it is surrounded by a gel-like membrane or envelope several molecules thick, consisting of a complex of lipid and protein.<sup>30</sup> Thus it is possible that cholesterol in combination with other lipids and the stromal proteins plays some part in determining the properties of the intact cell, though what properties are affected is not known. No relation was found between the cholesterol content of a cell and its osmotic resistance (Williams and co-workers) nor between the lipid content and the permeability to lipid-soluble substances (Dzemian). The fact that the cholesterol concentration is constant and the fact that the lipid is probably entirely in the free form, is independent of fluctuations in the plasma cholesterol and is associated with the stroma indicate it has no metabolic function in the red cell but has a structural role in combination with proteins and the other lipids.

The other cellular component of the blood, the leukocytes, contains much more lipid than the red cells. Boyd (1933 b), in a study of the white blood cell lipids of 8 normal young women, found that the con-

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29. Brun. Erickson and others.

30. Dzemian. Erickson and others.

centration of total lipid in these cells varied between 1 and 3 per cent, about four times their concentration in the plasma. The lipid fraction is composed of 47 per cent phospholipid, 31 per cent neutral fat and 11 per cent each of cholesterol and cholesterol esters. In general, the range of variation of these fractions was considerable. The white cells resemble body tissues, such as the heart, liver and brain, in their high total lipid and a relatively large proportion of phospholipid but resemble the plasma in their high content of cholesterol esters.

#### SUMMARY

The concentration of cholesterol in the serum of normal human beings of both sexes averages about 200 mg., with a range of from approximately 100 to almost 400 mg., per hundred cubic centimeters. Physicochemical studies of the blood serum indicate that the cholesterol is at least partially combined with the serum proteins and possibly other lipids in a colloidal complex, the structure and the composition of which are as yet unknown. In spite of the wide variance in the total cholesterol, the proportion of the free in the total cholesterol fluctuates within narrow limits, averaging about 30 per cent. This ratio is probably maintained through the continuous and simultaneous esterification of free cholesterol and hydrolysis of the esters.

Except for a pronounced rise during the first few days of life and a slight rise during childhood, the cholesterol level remains constant. A steady increase in the cholesterol with age in the absence of any metabolic abnormality has been reported by some investigators, but this has been disputed by others. The evidence indicates that during adult life the cholesterol of an individual tends to maintain a constant value uninfluenced by environmental conditions or living habits.

Though many contradictory reports have been published regarding the effect of diet on the serum cholesterol, certain conclusions may be drawn. Long periods of fasting produce no significant changes in the blood levels. Similarly, the character and the quantity of food ingested, whether taken over long or short periods, have little effect on the concentration of cholesterol. Though several authors have observed alimentary hypercholesteremia, particularly after the feeding of meals high in fat, the increases were not striking. Indeed, many investigators have been unable to detect hypercholesteremia even after the feeding of meals rich in cholesterol. It is noteworthy that when significant elevations of the cholesterol level occurred after the ingestion of this substance, the amounts fed were so large as to be unphysiologic. Apparently, in man hypercholesteremia normally does not develop as a result of the ingestion of moderate quantities of cholesterol.



## II. CHOLESTEROL METABOLISM

The previous section disclosed that the concentration of cholesterol in the blood of any person remains at a constant level, not easily changed by a diversity of physiologic conditions. In the body, cholesterol is constantly or intermittently synthesized and destroyed, absorbed and excreted, mobilized from and deposited in the tissues. The level therefore represents a dynamic balance between synthesis, absorption and mobilization, which tend to add cholesterol to the blood stream, on the one hand, and destruction, excretion and deposition, which tend to remove it, on the other. Any change in the level of the blood cholesterol, therefore, is a result of a disturbance in one or more of these processes. The proportion of free to esterified cholesterol also represents a dynamic equilibrium between continuous esterification and saponification in the blood or the tissues. Present knowledge of these interrelated and probably interdependent processes will be reviewed briefly.

## SYNTHESIS

Because of the polyterpenoid nature of the cholesterol molecule and the ubiquitous occurrence of terpene structures in plants, in contrast to their rarity in the animal kingdom, early chemists were reluctant to admit that animals were able to synthesize these compounds, and (on the mistaken notion that cholesterol and plant sterols were isomeric) believed that cholesterol was entirely exogenous in origin. These early views were expressed by Gardner and his collaborators (Ellis and Gardner, 1909).

The first conclusive evidence for the synthesis of cholesterol was reported in 1920 by Gamble and Blackfan. In balance experiments on infants they found a greater amount of sterol excreted than was ingested. Since then, this concept has been verified by a variety of studies on man and animals.<sup>31</sup> Even in pregnant women, in whom there is a large requirement of sterol for the growing fetus, a negative sterol balance was observed (Kaufmann and Muhlbock, 1933). Schoenheimer and Breusch, in experiments on mice, found the amount of sterol synthesized to be dependent on the quantity available in the food. With ingestion of large amounts of cholesterol, destruction, not synthesis, occurred.

Herbivora, which have no exogenous supply of cholesterol, must synthesize as much as the body needs. "Schoenheimer and co-workers (Schoenheimer, 1931) by systematic investigations showed that the sterols of plants cannot serve as a source of cholesterol, for they are not absorbed from the animal intestine. Even if phytosterols were absorbed in sufficient amount, their different carbon skeleton would render their transformation into cholesterol extremely unlikely.

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31. Beumer and Lehmann. Channon. Gardner and Fox, 1921. Heinlein. Kaufmann and Muhlbock, 1933. Randles and Knudson, 1925. Schoenheimer, 1929 d

Relatively little is known of the mechanism or the site of the synthesis of cholesterol. Such lower plants as the yeasts and the molds produce ergosterol when the only sources of carbon are sugars, fatty acids or sodium acetate (Bills). Minovici mentioned oleic acid as the precursor of cholesterol in the animal body; the evidence, however, is not convincing. Eckstein and Treadwell<sup>32</sup> suggested that fatty acids play a part in the synthesis of cholesterol. They observed that rats synthesized more cholesterol (as determined by the difference between dietary and fecal sterols) on a high fat than on a low fat diet. The excess was not due to depletion of the cholesterol of the body. There was, moreover, greater synthesis with fats of high than with fats of low iodine number. Discherl and Traut, however, failed to observe an increase in the synthesis of sterols in mice fed esters of stearic, oleic and linoleic acids over a control group fed a basic diet of oats.

Perhaps the best evidence against the conversion of fatty acid to cholesterol is found in experiments employing deuterium (heavy hydrogen) as a tracer. Schoenheimer and Rittenberg<sup>33</sup> administered small quantities of deuterium-containing water to mice until the body water contained 1.5 per cent; this concentration was maintained for varying periods. The concentration of deuterium in the body cholesterol slowly rose to one-half the concentration of that substance in the body water and remained constant. This important fact indicates that in the formation of cholesterol at least half of the hydrogens are exchangeable, a circumstance which could arise only by the coupling of a number of small molecules. The recent results of Sperry, Waelsch and Stoyanoff point in the same direction. In rats fed deuterium-containing fatty acids, there was no measurable uptake of deuterium in the tissue sterols.

On purely chemical grounds the direct conversion of fatty acids to cholesterol is unlikely. The polyterpenoid structure of cholesterol is so different fundamentally from the unbranched carbon skeleton of the fatty acids that any conversion of the latter to the former would be impossible without a complete breakdown of the fatty acid molecule.

The close structural similarity between cholesterol and other substances of biologic origin, such as squalene and vitamin A, has led to some interesting theories regarding the possible role of these substances as precursors,<sup>34</sup> but there is little conclusive evidence pro or con. The weight of evidence favors a mechanism in which cholesterol is built up of small units rather than by direct rearrangement of large molecules.

Though almost every organ has been suggested as the site of the synthesis of cholesterol in the body, little is known in this regard. The

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32. Eckstein and Treadwell, 1935, 1938. Treadwell and Eckstein, 1939.

33. Rittenberg and Schoenheimer. Schoenheimer, Rittenberg and Graff.

34. Bryant. Vanghelovici.

liver and the adrenal glands, because of their high cholesterol content, have been most frequently considered. The theory that cholesterol is synthesized in the adrenal glands was cited by Grigaut <sup>35</sup> on the basis of chemical and histologic studies of disease of the adrenal glands and especially in view of the previous observation <sup>36</sup> that hypercholesteremia develops in dogs and rabbits after unilateral adrenalectomy. He regarded the hypercholesteremia as a result of compensatory hyperactivity of the remaining gland. The studies of Baumann and Holly (1923) on rabbits and of Randles and Knudson (1928) on rats effectively refuted this postulate, as neither the former nor the latter investigators observed any changes in the blood cholesterol following unilateral or bilateral adrenalectomy.

The remarkable increase in cholesterol ester content of the adrenal glands of rabbits on a high cholesterol diet <sup>37</sup> suggests that these glands may function as a depot. At any rate, the cholesterol content of the adrenals appears to reflect the changes in the blood. Ewert (1935) has shown that in aseptic or infectious hyperthermia the decrease in the blood cholesterol is paralleled by corresponding changes in the adrenals. The old suggestion of Aschoff that the adrenals play a part in the intermediary metabolism of cholesterol has added significance since the discovery of the steroid nature of the cortical hormones.

The position of the liver as the central laboratory of the body has led to its consideration as the site of the synthesis of cholesterol. Artom and Minovici claimed to have demonstrated synthesis of cholesterol in the liver during autolysis and perfusion, but recent autolytic experiments on liver by Sperry and Brand have not confirmed these results. Experiments of Sperry, Waelsch and Stoyanoff, in which deuterium-containing water was fed to rats, indicate that the liver is no more important for the synthesis of cholesterol than other tissues, for the amount of deuterium in the sterol fraction of the liver was no greater than in other parts of the body. The recent report of Winter suggests that the liver may play a part in the synthesis of cholesterol. In balance experiments on rats whose livers were damaged by carbon tetrachloride poisoning, the sterol "loss" was greater in the experimental than in the control group with normal livers.

Other organs, particularly the brain, the lungs and the spleen, have been considered as sites for the synthesis of cholesterol, but the evidence thereof is not convincing. At present Bills's statement must be accepted: "When all the facts are considered, it appears as likely as not that animal sterols, insofar as they are endogenous, originate in the cells in which they occur."

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35. Chauffard and others. Grigaut, 1913.

36. Grigaut, 1913. Rothschild, 1914.

37. Kay and Whitehead. Weinhouse and Hirsch.

## DESTRUCTION

It is generally agreed that cholesterol may be destroyed in an appreciable amount in the animal body, and the amount thus metabolized is a function of the quantity in the diet. Dam (1931) studying chicks, Menschick and Page cats and Page and Menschick (1932) and Cook (1937) rabbits observed negative sterol balances when cholesterol was fed. Breusch, extending the previous experiments of Schoenheimer and Breusch, found that whereas on a low cholesterol diet there was a synthesis of about 13 mg. per hundred grams of mouse per day, on a high cholesterol diet there was destruction of about 4.3 mg. These figures represent only the net loss or gain in cholesterol, i. e., the difference between synthesis and destruction. Presumably, the absolute amounts synthesized and destroyed are much higher than the balance figures indicate. Beumer and Fasold attributed the destruction of sterols on high cholesterol diets to bacterial decomposition in the intestine; the weight of evidence, however, is against such a hypothesis. Neither Bischoff nor Dam (1934) observed any destruction of sterols by incubation of feces or of intestinal contents. The fact, demonstrated by Breusch, that only absorbable sterol, i. e., cholesterol, had any influence on the sterol balance is a strong indication that destruction occurs in the tissues and not in the intestines.

For a long time no information was available regarding the breakdown products of cholesterol, but recent data furnish glimpses of the possible route of the metabolism of this lipid. Page and Menschick (1930 b), supported by Schoenheimer (1932), detected spectroscopically a substance characteristic of cholestenone in atherosclerotic arteries, where masses of cholesterol had remained for long periods. Haslewood isolated 7-hydroxycholesterol from ox liver, and the same or an isomeric substance was found in pregnant mares' serum by Wintersteimer and Bergstrom. Apparently the 7 position in cholesterol, which is susceptible to attack *in vitro*, is also active physiologically. Waelsch and Sperry separated the unsaponifiable matter of deuterium-fed rats and found the ketone and hydrocarbon fractions, as well as the cholesterol, high in deuterium, suggesting a close metabolic relation.

The multitude of sex and adrenal hormones with similar steroid ring systems suggests a common precursor which may well be cholesterol, though no direct physiologic relation has as yet been established. Similarly, cholesterol may be a precursor of the bile acids, though again there is no conclusive evidence for or against this theory.

## ABSORPTION

The outstanding characteristic of the absorption of sterols in the animal body is its remarkable specificity. In a brilliant series of papers, Schoenheimer (1931) and his co-workers showed that whereas chole-

terol is absorbed readily in all species the sterols of plant and various derivatives of cholesterol are not absorbed to any appreciable extent. On measuring the extent of absorption by analysis of liver, lymph and feces it was found that the plant sterols (stigmasterol, brassicasterol and sitosterol), the wool sterols (lanosterol and agnosterol) and the four saturated cholesterol derivatives (dihydrocholesterol, coprosterol and their epimers) were not absorbed. Allocholesterol, which differs from the parent substance only in the position of the double bond, was absorbed to a slight extent; this was attributed to the ready isomerization of this substance to cholesterol. Dam and Brun found that dihydrocholesterol, readily absorbed, after two weeks constituted 11 to 13 per cent of the body sterols. Page and Menschick (1930 c) showed that ergosterol may be absorbed to a small extent.

As is true of the other lipids, the mechanism of the transport of cholesterol across the intestinal wall is obscure. It has been demonstrated repeatedly that fats aid in the absorption,<sup>38</sup> but how is not known. The presence of bile also aids in the absorption of cholesterol. Loeffler, extending the observations of Schoenheimer, his conclusions being fully substantiated by Hummel, found that the fivefold increase occurring in the hepatic cholesterol of mice fed cholesterol rose to sevenfold and thirteenfold respectively when the cholesterol was supplemented with desoxycholic and cholic acid. These studies suggest that bile salts aid in the absorption of cholesterol through formation of choleic acids in the manner proposed by Verzár<sup>39</sup> for the fatty acids. It is noteworthy, however, that the greatest effects were obtained<sup>40</sup> with glycocholic and cholic acids, neither of which is able to form choleic acid complexes with cholesterol (Wieland and Sorge).

The question of whether cholesterol is absorbed in the free or the esterified form is still open, though evidence favors the latter. Frölicher and Süllmann, in analyses of thoracic lymph from rabbits, found a large increase in its cholesterol ester content and concluded that esterification had occurred during absorption. Brockett, Spiers and Himwich found in dogs during absorption of fat a large increase in the cholesterol content of the thoracic lymph. Since the source of this cholesterol most probably was the bile, in which it is entirely in the free form, esterification must have taken place during or before absorption into the lymphatics. Schoenheimer and Hummel found that when cholesteryl oxalate was fed to mice the cholesterol was deposited in the liver and the oxalic acid was excreted by the kidneys. Several alternative explanations refute the idea that this experiment constitutes

38. Cook, 1936. Loizides. Verzár and McDougall. Versé.

39. Verzár and Kuthy. Verzár and McDougall.

40. Hummel. Loeffler.

evidence that cholesterol is absorbed in the free form. For example, the oxalate may have been hydrolized in the intestine and the cholesterol esterified with fatty acids prior to absorption, or the oxalate may have been absorbed as such and hydrolized in the epithelial cells or even in the blood.

Analyses of lymph from the thoracic duct show that cholesterol enters the blood stream by this route rather than by the portal vein. Whereas increases in the cholesterol of the lymph have been demonstrated repeatedly<sup>41</sup> during the absorption of cholesterol, the changes in the portal blood are slight and of doubtful significance.<sup>42</sup> It is doubtful if significant increases in the cholesterol concentration of the portal blood could be demonstrated at the height of the absorption of cholesterol, even if all this went by way of the portal vein, if one takes into account the rate of blood flow, the rate of absorption, possible dilution of the portal blood by intestinal secretions and the limitations on the accuracy of the analysis. The absence of an increase in the cholesterol of the portal blood over that of the arterial blood, therefore, would not constitute evidence against this route being taken during absorption.

The apparent restriction of the absorption of sterols to cholesterol itself suggests that an enzymatic rather than a physicochemical mechanism is responsible. Recently Schramm and Wolff, having found a cholesterol-esterifying enzyme which is activated tremendously by bile salts in the pancreas, offered the following mechanism for the absorption of cholesterol: The fatty acids, set free by pancreatic lipase, are combined with cholesterol under the influence of pancreatic cholesterol esterase and bile; in this form cholesterol is transported into the epithelial cells of the intestine. Here they again are split by the cholesterol-hydrolizing enzyme which Klein found abundantly present in intestinal mucosa. Based on the synergetic action of pancreatic enzymes and bile, this theory explains the necessity for fat and bile in the absorption of cholesterol and is in accord with the known facts, but as yet it has not been put to experimental test.

#### EXCRETION

Though some sterol is lost by the body in the secretions of the skin (Hueck) and possibly in the urine,<sup>43</sup> the greatest amount is excreted into the intestinal tract. The early views, discussed by Campbell, emphasized the bile as the source of the fecal sterol, but Sperry (1927) found no decrease in the unsaponifiable matter of the feces of dogs

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41. Brockett and others. Frölicher and Süllmann.

42. Boyd, 1936 g. Schally. Yuasa.

43. Butenandt and Dannenbaum. Gardner and Gainsborough, 1925.

with biliary fistulas. His results were substantiated by Beumer (1923), Beumer and Hepner (1929), Heinlein (1933) and many others, all of whom observed normal sterol balances with total exclusion of the bile from the intestine. In the feces of a patient with complete closure of the bile duct caused by carcinoma, Bürger and Winterseel (1929 a) found large quantities of sterols which could not be accounted for by the food eaten.

With elimination of the bile as an important source of the fecal sterols, several other possibilities suggest themselves: desquamation of intestinal epithelium, synthesis by intestinal bacteria and direct excretion from the blood. The first theory was examined by Bürger and Oeter. They found the sterol content of the intestinal mucosa extraordinarily low—so low that on a sterol-free diet the excretion of sterols is greater than the amount in the entire mucosa. Similar results were reported by Sperry (1929 c).

The possibility that fecal sterols arise through the synthetic action of intestinal bacteria was suggested by Sperry (1929 b, c), who found appreciable quantities of sterol in fecal bacteria separated by centrifugation. The possibility of adsorption makes the results inconclusive. The absence of sterols in bacteria, shown by various authors,<sup>44</sup> would indicate that they are not products of bacterial metabolism. Bürger and Oeter obtained indirect evidence for the direct transport of cholesterol into the intestinal lumen; in an analysis of segments of intestine, the sterol content of the sigmoid was found almost double that of the duodenum and ileum. The colon, therefore, probably accounts for a large portion of the excreted cholesterol.

There is a difference between omnivora and herbivora in the amounts of sterol excreted. In the herbivora Ellis and Gardner (1912) and Schoenheimer (1929 c), have shown no apparent excretion of sterol, the fecal sterol being the same in amount and composition as the dietary phytosterols. In omnivora and carnivora, on the other hand, large amounts of sterols are in the feces, a greater amount than can be accounted for by the food ingested. In man, Gardner and Fox (1921) estimate the amount of sterol excreted daily to be about 0.6 Gm.—about twice the amount in the food eaten.

*Origin of the Saturated Sterols of the Feces.*—In omnivora, the fecal sterols, unlike those of the body, are composed mainly of coprosterol, with smaller quantities of the isometric dihydrocholesterol (betacholestanol) and cholesterol. The two isometric saturated sterols, dihydrocholesterol and coprosterol, may be obtained in the laboratory (the former directly, the latter indirectly) by reduction of cholesterol. It is natural to assume that they arise physiologically in the same manner,

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44. Anderson and Chargaff. Von Behring. Beumer and Hepner.

though direct conversion *in vivo* has not been demonstrated. The significance of these saturated sterols has received intensive investigation by Schoenheimer and his co-workers.

Dihydrocholesterol, first isolated from feces by Windaus and Ulbrig, comprises a small, but definite, amount of the sterols of the entire body; from 1 to 5 per cent (Schoenheimer, von Behring and Hummel). Schoenheimer and von Behring proved that dihydrocholesterol was excreted directly by the intestinal mucosa. They allowed the secretions of blind sterile loops of the large intestines of dogs to accumulate for several months. Analysis of the contents for sterols showed only dihydrocholesterol plus a small quantity of cholesterol. These authors also cited an instance of a woman with a blind loop of intestine. The sterile contents contained dihydrocholesterol as the only sterol. Schoenheimer has pointed out that as dihydrocholesterol cannot be absorbed from the intestine, whereas cholesterol is readily absorbed, the elimination of the two sterols in the proportions in which they occur in the blood would be followed by absorption of the latter and accumulation of the former. If this hypothesis is correct, with a proportion of 1 to 5 per cent of dihydrocholesterol in the blood sterols and a fecal content of 100 mg., no less than 2 to 10 Gm. of cholesterol would be excreted and reabsorbed daily.

The presence of coprosterol in the stools cannot be explained on the same basis, however, for this sterol has never been found in the body tissues. Since its discovery by Bondzynski and Humnicki in feces, the view has prevailed that it is produced by the reduction of cholesterol by the bacteria normally present in the large intestine. In spite of much work on this problem, the bacterial origin of coprosterol has not yet been proved conclusively. Attempts by Schoenheimer, von Behring, Hummel and Schindel and by Dam (1934), and also by Bondzynski and Humnicki, to reduce cholesterol *in vitro* through the agency of putrefactive bacteria were unsuccessful. On the other hand, Dam (1934) and Bischoff observed a slight increase in the saturation of the sterols when feces or colon contents were incubated aerobically or anaerobically. The old observation of Muller that a milk diet, presumably by altering the bacterial flora, inhibits the formation of coprosterol and causes the appearance of cholesterol in the feces often has been quoted in support of bacterial reduction of cholesterol in the intestine. Bürger and Winterseel (1929 b) have not been able to corroborate this hypothesis. They, however, in an investigation of the effects of diet on the composition of fecal sterols, found a fairly constant ratio of cholesterol to saturated sterols in the feces of normal human beings, independent of the amount of cholesterol ingested and of dietary factors which would affect the bacterial flora. Dam's (1934) analyses of fecal sterol show also that the composition is independent of the diet.



The colon unquestionably is the only part of the intestine in which coprosterol is produced. Gardner, Gainsborough and Murray could not isolate coprosterol from intestinal contents discharged from a terminal ileostomy or a colostomy, and Dam (1934) reported the virtual absence of saturated sterols from ileal and jejunal contents.

The chemical mechanism of the reduction of cholesterol to coprosterol has been partially illuminated by Schoenheimer, Rittenberg and Graff through the application of the deuterium indicator method. When added to the basic meat diet of a dog, cholestenone, a direct oxidation product of cholesterol, is converted into coprosterol. If added to a basic diet of dog biscuit, however, it is reduced to cholesterol. When coprostanone, into which deuterium was incorporated, was administered to dogs and man, coprosterol containing deuterium was recovered in the feces. The physiologic significance of these ketones, though never isolated from body tissues and feces, appears to be established.

#### DEPOSITION AND MOBILIZATION

Though many of the studies of the deposition of cholesterol in body organs have emphasized the pathologic character of this process, evidence indicates that in the liver at least storage of cholesterol is a normal physiologic response to cholesterol overfeeding. The administration of this sterol in small, though greater than normal, amounts of the order of 1 to 2 per cent of the diet causes the deposition of considerable amounts in the liver of rats,<sup>45</sup> cats (Dam, 1934), rabbits<sup>46</sup> and chicks (Sperry and Stoyanoff, 1935 a). Practically all of the increase is confined to the esterified cholesterol, but the free cholesterol increases somewhat if the feeding is protracted (Chanutin and Ludewig, 1933). The storage of cholesterol is greatly enhanced by the simultaneous administration of large quantities of fat, owing probably to the effect of the latter on its absorption. The action of bile salts, previously pointed out, in enhancing the deposition of cholesterol in the liver may be attributed to increased absorption from the intestine.

Opinion differs regarding the quantitative effect of fat on the deposition of cholesterol. Whereas Loizides reported that the deposition of cholesterol ester was proportional to the amount of fat with which the cholesterol was incorporated, Cook (1937) found no greater storage of cholesterol with a 30 per cent fat diet than with one that was 15 per cent fat. Chanutin and Ludewig (1933) observed a greater deposition of cholesterol on a low fat, high carbohydrate diet than on a high fat, low carbohydrate diet.

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45. Channon and Tristan. Chanutin and Ludewig. Cook, 1937. Loizides.

46. Aylward and Stott. Dam, 1934. Weinhouse and Hirsch.

Okey, Gillum and Yokela claimed to have found a difference in susceptibility to cholesterol feeding between males and females. The slightly lower cholesterol content of the liver in female than in male rats was statistically significant.

A new field of investigation in lipid metabolism has been opened in recent years with the discovery that extraordinarily large amounts of fat are deposited in the livers of rats on certain diets, a condition that can be prevented or cured by the administration of various lipotropic substances, of which choline is probably the most outstanding example. As this subject was reviewed recently by Best and Ridout (1939), Channon (1940) and Dragstedt, only the portion of the work related to cholesterol metabolism will be reviewed here.

In contrast with the "fatty" livers produced with high fat, high carbohydrate, low protein or low choline diets or with starvation, in which the changes in the cholesterol content are hardly significant, the "fatty" livers observed after cholesterol feeding have not only a high glyceride content but also considerable quantities, as much as 6 per cent or more of the fresh tissue, of cholesterol esters.

Application of the lipotropic factors, effective in fatty livers of the first type, to the cholesterol fatty liver revealed that the cholesterol esters, as well as the glyceride, were decreased. This effect was obtained with choline,<sup>47</sup> betaine (Dam, 1934), casein (Beeston and co-workers), methionine (Channon, Manifold and Platt) and various analogues of choline (Channon, Platt and Smith). The effect on the cholesterol esters was not so great, however, as on the glyceride fraction. In contrast with the work carried out on rats as a test species, it is of interest that Baumann and Rusch were unable to prevent the deposition of cholesterol in the livers of rabbits by means of choline. It is noteworthy that lecithin exerts the same lipotropic effect as its choline content (Best, Hershey and Huntsman), which might suggest that the lipotropic effect of choline is due to increased synthesis of phospholipid. The apparent constancy of the phospholipid content of "fatty" livers, however, refutes this hypothesis. Yet the studies of Chaikoff and his group, using radioactive phosphorus as an indicator, have shown that the rate of the turnover of phospholipids in the liver is increased by the lipotropic factors, choline, betaine and methionine (Perlmann, Stillman and Chaikoff), and inhibited by cholesterol (Perlmann and Chaikoff). This experimental proof favors the suggestions previously made that the phospholipids play a part in the deposition and the mobilization of fat.

Another type of fatty liver develops in dogs which have been depancreatized and maintained on insulin or in which the pancreatic duct has

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47. Beeston and others. Best, Channon and Ridout. Best and Ridout, 1935, 1936. Dam, 1934. Stoesser, McQuarrie and Anderson.

been ligated. The condition of the liver resembles that obtained on diets low in lipotropic factor; i. e., there is a very high glyceride content with little or no change in the cholesterol or the phospholipid content.<sup>48</sup> The condition may be cured or prevented with choline or a lipotropic factor in pancreas (the lipocaic of Dragstedt). According to Chaikoff, the development of "fatty" liver through pancreatic deficiency is accompanied by a decrease in the cholesterol esters of the blood to vanishingly low levels.<sup>49</sup> Such changes in the blood have not been observed in other cases of lipotropic deficiency. The blood lipid levels returned to normal with ingestion of pancreas or choline, though much larger amounts were needed than for removal of the hepatic fat (Entenman, Chaikoff and Montgomery).

*Differences in Cholesterol Deposition Between Omnivora and Herbivora.*—In omnivora, of which the rat has been the best studied example, the deposition of cholesterol is confined almost completely to the liver.<sup>50</sup> The most thorough study has been carried out by Okey and her co-workers. They found that in spite of the intensely fatty livers of these animals, containing more than twenty times the normal quantity of cholesterol, no effect on the general health was observed. Rats fed 1 per cent of cholesterol withstood poorly balanced and vitamin-deficient diets almost as well as the controls (Gillum and Okey). Pregnancy and lactation were successful (Okey, Godfrey and Gillum), and a group of rats, fed the high cholesterol diet throughout their entire lives, grew as well, lived as long and remained as healthy as control animals (Okey, 1941). Sperry and Stoyanoff (1935 b) studied the effects on rats of long-continued feeding of diets containing 1 per cent cholesterol. The rats receiving a "synthetic" diet containing cholesterol grew less well, ate less food and utilized their food less efficiently than controls receiving the synthetic diets without cholesterol. Such differences were not observed if a diet of natural foods was given in addition to the cholesterol. Undoubtedly, the synthetic diet lacked the lipotropic factors present in the natural foods.

Herbivora, on the other hand, have a poor tolerance for cholesterol. In response to its inclusion in the diet of rabbits in amounts of less than 1 per cent, deposition occurs in all parts of the body, particularly the liver, the adrenal glands and the aorta. Hardly any tissue of the body remains unaffected, deposits of cholesterol occurring in the corneas, kidneys, lungs, spleen, gonads and bone marrow (Schoenheimer, 1924 a).

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48. Aylward and Holt. Kaplan and Chaikoff.

49. Entenman, Chaikoff and Montgomery. Entenman, Montgomery and Chaikoff.

50. Chanutin and Ludewig. Page and Menschick, 1933. Schoenheimer, 1924. Sperry and Stoyanoff, 1935 b.

The reason omnivora may tolerate large quantities of cholesterol without their health being affected in any manner may be that the hepatic cells have the ability to store and probably to destroy large amounts of the substance without damage to the organ (Page, 1941). The demonstration of active excretion of sterols in omnivora (Schoenheimer and von Behring) coupled with the apparent lack of excretion of cholesterol in rabbits and other herbivora<sup>51</sup> may well account for their differences in toleration of exogenous cholesterol.

The metabolism of cholesterol may be summarized briefly as follows: Exogenous cholesterol is absorbed from the intestine with the aid of fats and bile. It passes by way of the lacteal and the lymph ducts into the general circulation, where part is deposited with the depot fat and part enters the liver to be stored, excreted or destroyed. In omnivora the main route of the excretion of cholesterol is the intestine, where it may be reabsorbed or reduced to coprosterol, the form in which it is found in the feces. No excretion of sterols has yet been demonstrated in herbivora except that of the bile, which probably is completely reabsorbed, and that of the small quantities secreted through the skin. The synthesis and the destruction of cholesterol proceed actively in omnivora and herbivora, the relative rates of each process depending on the available dietary cholesterol. The fact that omnivora tolerate large amounts of ingested cholesterol, whereas herbivora show the most widespread effects, is due either to difference in the relative abilities of their livers to store it or to difference in their ability to excrete it.

### III. BLOOD CHOLESTEROL IN DISEASE

#### DISEASES OF THE LIVER

The changes in the cholesterol of the blood in hepatic and biliary disease have received thorough investigation, especially from the clinical standpoint, and though investigators agree essentially in their observations, they differ in their interpretations.

Thannhauser and Schaber, using reliable methods, were the first to observe a diminution in the ratio of esterified to free cholesterol in the blood of patients suffering from disease of the liver. The wealth of early clinical data published since then is confusing because of the variety of analytic methods and because of the inclusion of cases complicated by other diseases. The early work was reviewed by Gardner and Gainsborough (1930 b) and Epstein (1932).

The more recent studies agree that in parenchymatous hepatic disease the serum cholesterol esters reach low levels. Epstein (1937), in a study of 130 cases of jaundice associated with diffuse parenchymal damage due to drugs and other causes, with no biliary obstruction, found

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51. Ellis and Gardner, 1912. Schoenheimer, 1929 c.

normal or low values for the total blood cholesterol in 88 per cent. A decrease in the cholesterol esters in 70 per cent paralleled the severity of the clinical symptoms.

Lowered levels of blood cholesterol esters were especially marked in 15 cases of acute, subacute and chronic yellow atrophy. In each, the esters remained low throughout the entire fatal course, at times almost vanishing. The behavior of the blood cholesterol in 35 cases of atrophic cirrhosis is interesting. In 24 cases in which jaundice was absent the ester cholesterol was diminished only slightly, whereas in 11 cases with jaundice the changes in the cholesterol esters resembled those in cases of primary degeneration of the liver.

Similar values for blood cholesterol in cases of hepatic damage were reported by many others.<sup>52</sup> Boyd and Connell (1938) found the lowering of cholesterol esters in nonobstructive jaundice to be only a part of general lipopenia. In 27 cases of parenchymal disease there were, in addition to the lowering of the cholesterol esters, marked decreases in the plasma phospholipids, with slight decreases in the free cholesterol and the neutral fat. Coincident with the lipopenic changes in the plasma, these authors observed increases in the lipid content of the red cells, though not consistently enough for the increases to be statistically significant.

Experimental studies of hepatic injury corroborate the clinical findings. Hawkins and Wright, in dogs with chronic injury of the liver produced by chloroform, observed a drop in the plasma cholesterol esters which paralleled the jaundice and the severity of the clinical condition. When the ester cholesterol dropped to less than 30 per cent of the total, the animals were critically ill, and died when values fell to between 0 and 26 per cent. With subsidence of the jaundice and recovery, the cholesterol esters again became normal or slightly higher. Yet acute injury of the liver from one hour of chloroform-induced anesthesia, accompanied by marked bilirubinemia and the usual toxic symptoms, caused no change in the total cholesterol or in the percentage of esters. Kusui observed a decrease in the blood cholesterol esters after poisoning with toluylendiamine and a return to normal with recovery. In the studies of Chanutin and Ludewig in rats after partial hepatectomy (65 to 75 per cent) the blood cholesterol esters were markedly lower in twenty-four hours but normal after three days. As it was probable that little regeneration had taken place in this time, the authors concluded that the decrease was caused not by removal of the tissue but by dehydration or other damage of the hepatic remnant owing to the shock of the operation.

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52. Beumer, 1936. Greene and others. Kusui. Pickhardt and others. White, Deutsch and Maddock.

In contrast to jaundice from damage of the liver, the jaundice associated with biliary obstruction is accompanied by hypercholesteremia (over 300 mg. per hundred cubic centimeters) in 78 per cent of patients in whom the condition was verified at operation or at autopsy. There was no marked correlation between the blood cholesterol and the icteric index, although with relief of the obstruction and diminution of the jaundice the cholesterol was reduced to normal.<sup>53</sup>

Hawkins and Wright observed a marked rise in the total cholesterol but no change in the ratio of free to ester cholesterol in dogs whose bile ducts were ligated. Similar results were reported by Chanutin and Ludewig in rats with ligated bile ducts.

As the ratio of free to total cholesterol normally fluctuates between 25 and 30 per cent, any marked increase in the ratio accompanied by jaundice is strongly suggestive of hepatic damage. This test successfully distinguishes between jaundice of obstructive origin, in which hypercholesteremia with no significant change in the ratio of free to ester cholesterol is found, and the jaundice associated with parenchymal injury, in which the total plasma cholesterol is normal or low and the ratio of free to total cholesterol is increased.<sup>54</sup> Though Bürger (1940) tends to minimize the relation between the partition of cholesterol and hepatic disease, his figures afford good evidence in favor of the relation. It is surprising that this simple test has not been more generally adopted by clinical laboratories. The proportion of free to ester cholesterol is of value in prognosis as well as in differential diagnosis. A continuously decreasing percentage of cholesterol esters indicates severe injury with a bad prognosis; a rising percentage indicates recovery (Greene and co-workers). Because of the multiplicity of hepatic functions, probably no single test will give a reliable picture of this organ, but the cholesterol ester ratio in combination with other clinical and laboratory data is a valuable aid.

Thannhauser and Schaber interpreted the decrease in cholesterol esters in hepatic disease to be a result of inability of the injured cells of the liver to esterify cholesterol, a phenomenon they named *Estersturz*. Many subsequent investigators have agreed, yet there is no conclusive evidence for this hypothesis. Thannhauser claimed to have found a cholesterol ester-synthesizing enzyme in liver. The results of Sperry and Brand, however, were contradictory. They found that an emulsion of rat liver esterified free cholesterol but that the same emulsion incubated with rat serum caused hydrolysis of cholesterol esters. Gardner and Gainsborough (1930 b) expressed the belief that the lower-

53. Thannhauser and Schaber. White, Deutsch and Maddock.

54. Boyd and Connell, 1938. Epstein, 1937. Greene and co-workers. Kusui. White, Deutsch and Maddock.

ing of cholesterol esters may be attributed to the absence of bile from the intestine, which results in nonabsorption of fats and cholesterol. This contradicts several experimental facts: First, as pointed out in the previous section, there is no significant relation between the amount of cholesterol in the diet and the level of cholesterol in the blood; second, nonobstructive jaundice, in which there is no absence of bile from the intestine, causes a decrease in cholesterol ester, whereas complete biliary obstruction actually increases blood cholesterol. Another theory rests on a firmer foundation. Beumer (1935, 1936) found large amounts of cholesterol esters in the livers of patients with acute yellow atrophy and concluded that the decrease in cholesterol esters of the blood in jaundice is a result of their fixation in the diseased liver. No marked variation in cholesterol content between normal livers and those which might have been associated with a decrease in ester cholesterol was noted, however, in a study by Ralli, Rubin and Rinzler or in one by Ralli, Paley and Rubin.

At present the explanation of the marked reduction of blood cholesterol esters in hepatic disease points to the inability of the hepatic cells to synthesize cholesterol esters or to transport them into the blood.

No satisfactory explanation exists for the hypercholesteremia associated with biliary obstruction. Early investigators regarded it as merely a mechanical effect of the obstruction, which impaired excretion of cholesterol. With the finding that the bile cholesterol is completely absorbed, however, and that the main pathway for the excretion of cholesterol is the lower intestine, this explanation does not suffice.

*Problem of the Gallstones.*—The formation of gallstones was attributed in former days to a disturbance of the metabolism of cholesterol, conforming with the prevailing belief that cholesterol of alimentary or endogenous origin was excreted exclusively with the bile. Hypercholesteremia, it was assumed, resulted in an overburdening of the excretory mechanism, with supersaturation of the bile and consequent precipitation of cholesterol and formation of a concrement.

This theory was questioned when Sperry (1927) demonstrated that the intestine, not the bile, is responsible for the excretion of sterols. The theory had to be abandoned after Campbell, Fox, Gardner and Gainsborough (1930 f) and Dostal and Andrews demonstrated conclusively the lack of any relation between dietary cholesterol or hypercholesteremia and the concentration of cholesterol in the bile.

About ten years ago, Andrews and associates<sup>55</sup> suggested a mechanism for the formation of gallstones which has stood the test of time and been confirmed amply by subsequent research. The formation of

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55. Andrews, Dostal, Goff and Hrdina. Andrews, Dostal and Hrdina. Andrews, Hrdina and Dostal. Andrews, Schoenheimer and Hrdina. Dostal and Andrews.

stones, according to this hypothesis, results from a decrease in the ratio of bile salts to cholesterol in the bile. Andrews demonstrated that cholesterol ordinarily is dissolved in the bile through the influence of the bile salts (Andrews, Schoenheimer and Hrdina), probably by formation of loose compounds. This was confirmed subsequently by Spanner and Baumann<sup>56</sup> and by Bashour and Bauman. Of the bile acids present in bile, the best solvent for cholesterol appeared to be desoxycholic acid.

In various species of animals (goats, rabbits, cattle and dogs) the ratio of bile salts to cholesterol was about 100 (Wright and Whipple); in man, it was only 20 to 30. The fact that the critical ratio, below which cholesterol begins to precipitate, is 13 may explain why cholesterol stones are relatively common in man and are apparently absent in animals. A decrease to one-half the normal ratio in the former would lead to precipitation, absent in the latter until an eightfold decrease has occurred.

In studying the factors which might influence the ratio of bile salts to cholesterol, Andrews and associates<sup>57</sup> found that the normal gallbladder does not absorb or excrete bile salts or cholesterol. The absorption of fluid which normally occurs is unaccompanied by any change in the ratio of bile salts to cholesterol or in the ratios of either of these substances to the total solids of the bile. Riegel and co-workers (1931) provided further evidence that neither bile salt nor cholesterol is absorbed or excreted from the healthy gallbladder.

Elman and Taussig and Wilkie and Doubilet claimed to have shown that cholesterol is secreted normally by the mucosa of the gallbladder, but Riegel attributed their results to errors in experimental procedures.

When the gallbladder is injured or inflamed, the wall becomes permeable to bile salts, as shown by Andrews, Schoenheimer and Hrdina; this results in a striking decrease in the ratio of bile salts to cholesterol with a consequent tendency toward precipitation of the latter. This was confirmed by Riegel, Ravdin and Johnston, who observed that dilution rather than concentration of bile occurred as a result of injury to the gallbladder. Bashour and Bauman showed that dilution lessened the solvent effect of the bile salts on cholesterol.

If cholesterol were excreted by the mucosa of the gallbladder, there should be an accumulation of this substance in long-standing cases of obstruction of the cystic duct. In this condition, the bile salts are absorbed, and the cholesterol is usually in the form of a crystalline emulsion; the amount present, however, is no more than can be accounted for by precipitation of the cholesterol in the bile ordinarily present. In fact, the stones found in the obstructed gallbladder usually are com-

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56. Pickens, Spanner and Baumann. Spanner and Baumann.

57. Andrews, Dostal and Hrdina. Andrews, Schoenheimer and Hrdina.



posed of calcium carbonate rather than of cholesterol. As Phemister and co-workers have demonstrated, if cholesterol stones are already present, obstruction and bile stasis cause additional precipitations of calcium salts. The foregoing evidence established the role of cholecystitis in the formation of gallstones; yet Andrews, Hrdina and Dostal suggested another factor: that is, the marked lowering of the ratio of bile salts to cholesterol in the hepatic bile in mild disease of the liver.

The correctness of Andrews' views is corroborated by the finding by Andrews, Schoenheimer and Hrdina and by Neumann that the ratio of bile salts to cholesterol in the bile of the gallbladder with concretions is lowered. Doubilet and Colp made similar observations in chronic and acute cholecystitis.

Cholecystitis and hepatic disease, then, appear to help alter the ratio of bile salts to cholesterol, with subsequent precipitation of the latter.

Some investigators have attempted to show that other substances, such as the fatty acids, are important in holding the bile cholesterol in solution. For example, Dolkhart, Lorenz, Jones and Brown, in experiments completely unphysiologic as to  $p_H$  and temperature, purported to prove that fatty acids exert a marked solvent effect on cholesterol, being more important than the bile acids in this respect. These investigators, however, have not shown any appreciable solvent effects of fatty acids or their soaps on cholesterol under physiologic conditions. More important, they have not shown that fatty acids are present in normal bile.

Though the factor which determines whether or not cholesterol will be precipitated appears to be the ratio of the substance to the bile acids, the subsequent formation of the concretion depends undoubtedly on a multitude of factors which are not clearly understood at present.

#### RENAL DISEASE

A survey of the quantitative studies of blood lipids in Bright's disease reveals that high blood cholesterol is characteristic of true nephrosis and the chronic, nephrotic stage of nephritis. Page, Kirk and Van Slyke, in a study of 13 cases of hemorrhagic nephritis, found that the chronic stage of this disease is accompanied by hyperlipemia, involving all the blood lipids. Repeated determinations showed no change in the proportion of each lipid. Similar findings in nephritic patients were obtained by Page and Farr. These investigators contended that nephritic lipemia is uninfluenced by the amount of fat in the diet and, unlike the lipemia associated with myxedema, is not reduced by the feeding of large quantities of thyroxin. They found the ratio of free to total cholesterol unchanged, contrary to the earlier results of Lichtenstein and Epstein, who reported an increase in the proportion of esterified cholesterol in nephrosis and glomerular nephritis.

The origin of nephritic hypercholesteremia and lipemia is obscure. The view sometimes has been expressed that nephrosis is primarily a result of disturbance in lipid metabolism. That similar blood lipid pictures are found in conditions such as nephrosis and glomerular nephritis, which differ distinctly in causation and pathogenesis but are similar as to tubular degeneration, hypoproteinemia and edema, suggests that lipemia is a result rather than a cause of the loss in normal renal function.

That the lipid level is reciprocally related to the protein concentration of the blood is probably of fundamental importance. Inasmuch as the plasma proteins are responsible for the colloidal stability of the lipids, a lowering of protein would presumably cause a decrease in the dispersion of the lipid complex, with resultant agglomeration into larger particles. The decrease in surface available for the action of the lipolytic enzymes hence would lead to a lowered rate of transport and utilization, resulting in accumulation of lipids in the plasma to the point of macroscopically visible lipemia. This crude explanation for nephritic lipemia, though speculative, is supported by the observation that in cardiac edema, which is not necessarily accompanied by hypoproteinemia, there is no increase in the blood cholesterol.<sup>58</sup>

Another explanation for the hypercholesteremia of renal disease has been suggested by Miyazaki. By injecting renal venous serum from a normal rabbit into another normal rabbit, he obtained a rapid fall in blood cholesterol, but a similar injection of arterial or auricular venous blood had no effect. This action was ascribed to a hormone secreted by the renal tubules. It not only lowered the blood cholesterol of the normal rabbit but markedly reduced the hypercholesteremia produced by experimental nephritis or nephrectomy. This study, indicating a hormonal disturbance in the lipemia of nephrosis, would be important if verified.

The deposition of granules of anisotropic lipid material (cholesterol esters) in nephrotic kidneys, giving rise to the term "lipoid nephrosis," is probably unimportant, as it resembles the precipitation of lipid substances in other degenerating tissues. Similarly, the cholesteroluria probably does not signify a specific permeability of the injured kidney to cholesterol, which most likely escapes with the protein to which it is normally bound in the plasma (Bruger, 1935 b).

#### DISEASE OF THE THYROID GLAND

Although the relationship between the function of the thyroid gland and the metabolism of cholesterol and other lipids is unknown, the changes in the blood cholesterol level associated with abnormalities of

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58. Bodansky and Bodansky. *Port.*

this gland are well established. There is general agreement, based on clinical and experimental studies, that high values for blood cholesterol accompany hypothyroidism and that low normal or subnormal values are found in hyperthyroidism.<sup>59</sup>

In myxedema Hurxthal found values up to 500 mg. and Gildea, Man and Peters values as high as 600 mg. per hundred cubic centimeters. These investigators claimed a fair correlation between the disappearance of clinical symptoms, the rise in the basal metabolic rate and the fall in blood cholesterol after thyroid therapy. The relation between the basal metabolic rate and the cholesterol level stressed by many investigators<sup>60</sup> is apparent only when the values in a large number of cases are averaged. When individual instances are examined, many exceptions are found (McGee), a not unexpected result in view of the dependence of the basal metabolic rate on factors other than thyroid activity.

In 21 children with hypothyroidism who were untreated, Wilkins, Fleischmann and Block observed serum cholesterol levels varying from 145 to 160 mg. per hundred cubic centimeters. In contrast with normal children, these children showed great spontaneous variations. The marked instability of the serum cholesterol in myxedema, these authors pointed out, may explain the low values frequently observed in this condition.

In myxedema produced by thyroidectomy and in the spontaneous variety, hypercholesteremia was reported by Gilligan, Volk, Davis and Blumgart; it was observed by Blumgart and Davis after removal of the thyroid gland in the treatment of chronic heart disease. Similar results were reported in cases of thyroidectomy for hyperthyroidism (Man, Gildea and Peters). The rise in the cholesterol concentration was variable, however, and appeared independent of the degree of recovery. The effect of thyroidectomy on the cholesterol level is well illustrated in animal experiments. According to Schmidt and Hughes, in normal dogs thyroidectomy produced marked hypercholesteremia, which reached a peak four to five weeks after operation and which was accompanied by no change in the normal ratio of free to esterified cholesterol and was confined to the plasma.

Most investigators find that in rabbits thyroidectomy is followed by hypercholesteremia but disagree as to the magnitude of this change. Turner, Present and Bidwell observed only a 19 per cent increase, but Westra and Kunde and Fleischmann, Schumaker and Wilkins found increases of about 200 per cent. The effect of thyroidectomy on rabbits with hypercholesteremia of alimentary origin was studied by Turner,

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59. Bodansky and Bodansky. Mason, Hunt and Hurxthal. Page, 1937.

60. Cutting, Rytand and Tainter. Epstein and Landé. Gardner and Gainsborough, 1928.

Present and Bidwell. They reported that thyroidectomy caused a 137 per cent rise in the blood cholesterol. In so-called cholesterol-resistant rabbits, moreover, thyroidectomy promptly abolished the resistance and hypercholesteremia promptly ensued.

Hyperthyroidism, according to Hurxthal (1933 b) and others, is accompanied by blood cholesterol levels which on the average are definitely below normal though there is much overlapping of normal ranges. Though studies, reviewed by Man, Gildea and Peters, have indicated normal, even high blood cholesterol levels in hyperthyroidism, nevertheless, the weight of evidence indicates that subnormal values are to be expected to accompany this disease.

In hypothyroidism the variations from normal cholesterol values are large, and small changes in the basal metabolism effect relatively great changes in the cholesterol level. Thus, blood cholesterol determinations in this condition are valuable both in diagnosis and in following the efficacy of treatment. They are especially useful in regard to childhood myxedema, in which the basal metabolic rate is often unstable.<sup>61</sup> In hyperthyroidism, on the other hand, cholesterol levels are almost invariably near or within the normal range and are not changed markedly by decreases in the basal metabolic rate. In this condition, therefore, blood cholesterol determinations probably have little clinical usefulness.

Boyd and Connell (1936) have shown that the hypercholesteremia of myxedema is part of a general hyperlipemia. Of 35 patients with low metabolic rates, 19 showed no improvement with thyroid medication. Their blood lipid levels were not significantly abnormal. Of the other 16, who all improved under thyroid therapy, the levels of blood lipids, fatty acids, free and total cholesterol and phospholipids were well above normal. In 43 patients with hyperthyroidism the same authors observed subnormal levels for all lipid fractions. Despite large individual variations, they concluded that 5 of 6 patients with hyperthyroidism would have lower lipid levels than 5 of 6 normal persons. In hypothyroidism (Gildea, Man and Peters) and hyperthyroidism (Man, Gildea and Peters) Man, Gildea and Peters found that the concentration of phospholipids and of fatty acids paralleled that of the cholesterol in the serum. During therapy the changes in the cholesterol levels were accompanied by proportionate changes in the levels of the other lipids.

The effect of the thyroid gland on the cholesterol and other lipids of the blood is unknown. The levels of these substances undoubtedly are markedly affected by the presence of thyroxin. Administration of whole thyroid or of thyroxin to myxedematous patients lowers the levels of serum cholesterol<sup>62</sup> and other lipids (Gildea, Man and Peters).

61. Görtz. Wilkins and others.

62. Hurxthal, 1933 b. Lahey.

Thyroxin also lowers the serum cholesterol in normal persons (Gildea, Man and Peters). The effect of the thyroid hormone is well illustrated in animals. In recent experiments Hughes showed that administration of thyroxin or of desiccated thyroid to thyroidectomized dogs reduced the plasma cholesterol if it was already high but had no effect in dogs whose blood cholesterol was low. Massive doses had no greater effect than moderate ones. The observed changes were confined to the plasma.

Because of the lability of the blood cholesterol in herbivora, the effect of thyroxin on the level of this component in rabbits is of interest. Thyroxin, even when administered in large doses, produced only slight decreases in the blood cholesterol of normal rabbits. Thyroidectomized rabbits are much more responsive to it. Administration of thyroxin to intact rabbits in which hypercholesteremia had been induced by cholesterol feeding caused a substantial drop in the serum cholesterol (Turner, Present and Bidwell). A single dose of 1 mg. of the crystalline hormone produced an average 40 per cent decrease. The minimum value was reached in three or four days, the level returning to its previous figure in five to nine days. In thyroidectomized rabbits with alimentary hypercholesteremia, the response differed only in degree, the drop averaging 60 per cent.

Despite the relation between the basal metabolic rate and the blood cholesterol, considerable evidence indicates that the action of thyroid on the blood lipids is not a direct consequence of its effect on metabolism. Hurxthal (1934) has shown that hypometabolism associated with deficiency of the adrenal glands or of the pituitary gland is not accompanied by hypercholesteremia. Large increases in basal metabolism caused by the administration of 2,4-dinitrophenol, furthermore, cause no appreciable drop in the blood cholesterol.<sup>63</sup> These observations suggest that the thyroid hormone does more than regulate the body metabolism. Its effect on the blood and tissue lipids awaits further study.

#### ATHEROSCLEROSIS

The early identification of cholesterol as a constituent of atheromatous lesions led to speculations regarding its importance in the causation of atherosclerosis. Some investigators have considered the nodular or diffuse accumulations of lipids in this disease as secondary to medial injury (Wells ; Duff) ; others, notably Aschoff, Anitschkow and Leary, have expressed the belief that the deposition of lipids precedes any other changes in the artery.

The influence of the amount and the character of the blood lipids on the development of atherosclerosis has been studied extensively with

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63. Cutting and co-workers. Emmer. Grant and Schube. Hurxthal, 1934.

the result only that there has been published much controversial data. Inasmuch as atherosclerosis occurs without definite symptoms until the late stages, the determination of its presence and extent in human patients is difficult. The relationship of the blood lipid levels to this disease can be inferred only by studying conditions, such as hypertension or angina pectoris, closely associated with atherosclerosis.

Mjassnikow (1925) found elevated blood cholesterol levels in angina pectoris, the values varying from 190 to 440 mg., compared with 120 to 170 mg., per hundred cubic centimeters for normal subjects. Davis, Stern and Lesnick reported blood lipid levels frequently elevated in 59 patients with angina pectoris. About 60 per cent had total blood cholesterol levels over 250 mg. per hundred cubic centimeters, whereas only 20 per cent of 54 normal persons had values this high. As a group the patients with angina pectoris had values averaging 260 mg. per hundred cubic centimeters, compared with 218 mg. for the controls. Statistical analysis indicated that the results were significant. Increase of phospholipids and fatty acids also was observed. The ratio of free to total cholesterol remained constant in normal and in diseased patients. In 73 patients with arteriosclerosis obliterans of the legs unassociated with diabetes or hypothyroidism, Barker found that the mean value for blood cholesterol was 263 mg., compared with 218 mg. for normal subjects. Statistical analysis was not carried out, but inspection of the data reveals that the significance of this difference is not great. Poin-dexter and Bruger observed high average values for the blood cholesterol in 24 cases of arteriosclerosis and in 19 cases of hypertension with arteriosclerosis. The average for these was 250 mg. per hundred cubic centimeters; 33 normal persons had an average value of 195 per cent—statistically a significant difference.

Other investigators have reported normal blood cholesterol levels in clinical conditions associated with arteriosclerosis. Andes, Kampmeier and Adams found no difference in cholesterol level between normal and arteriosclerotic Negroes. In 16 patients with hypertension uncomplicated by nephritis, Page, Kirk and Van Slyke (1936 b) found normal blood lipid levels. In no case was the concentration of any of the lipid fractions (free and total cholesterol, phospholipid and total lipids) outside the range of normal values, nor were the means and standard deviations different from those in normal subjects. In 53 patients with hypertension, Elliot and Nuzum found no significant increase of blood cholesterol. No correlation was noted between the severity of clinically determined arteriosclerosis and the level of the blood cholesterol.

Probably the most important study was made by Landé and Sperry. Recognizing the difficulties in measuring the extent of arteriosclerosis by clinical methods, they determined the postmortem blood cholesterol

of persons who had died suddenly by violence. After comparing the values thus obtained with the degrees of atherosclerosis ascertained by an objective method, namely, the determination of the lipid in the whole aorta, and eliminating all cases complicated by infection or organic disease, they concluded that no relation existed between the blood cholesterol level and the severity of atherosclerosis.

In many, if not most, cases atherosclerosis evidently is unaccompanied by hypercholesteremia. Although the relation of the blood lipids to the development of atherosclerosis remains undisclosed, there is hardly any doubt that the disease can develop in the absence of any abnormality in the levels of blood lipids.

Considerable evidence indicates that atherosclerosis is advanced by hypercholesteremia. The best and most direct proof is had from the results of experimental cholesterol feeding in rabbits. Here the relation of cause and effect is clear and unequivocal. Cholesterol when fed by mouth accumulates in the blood stream; then it is deposited in the aorta in atheromatous plaques closely resembling those of the human disease. Yet other factors are involved besides hypercholesteremia. Although atherosclerosis never was observed in normal rabbits and invariably was found in cholesterol-fed animals, Weinhouse and Hirsch found no relation between the duration and the height of hypercholesteremia and the degree of atherosclerosis. Differences undoubtedly are to be found in the receptivity of the tissue for the blood lipids. These differences also apply to different tissues of the same animal: One rabbit, for example, may have marked atherosclerosis without changes in other organs, and another have little or no atherosclerosis but marked infiltration of the liver or the kidneys.

The effect of factors other than hypercholesteremia in experimental cholesterol atherosclerosis in rabbits was shown by Page and Bernhard. The atherosclerosis following cholesterol feeding can be prevented by simultaneous administration of thyroid extract or organic iodides, their effect, according to Turner, being to prevent the development of hypercholesteremia. Page and Bernhard, however, found that an organic iodine compound having the same protective action against atherosclerosis as thyroid or inorganic iodides raised the blood cholesterol levels of cholesterol-fed rabbits to higher values than those of rabbits fed this substance alone. These iodine compounds evidently protect rabbits against atherosclerosis by affecting the receptivity of the tissue rather than by altering the blood cholesterol.

Clinical evidence that hypercholesteremia is a predisposing factor in atherosclerosis is the extraordinary prevalence of arteriosclerosis in diabetic patients. The close association between high cholesterol and arteriosclerosis in patients with diabetes has been demonstrated by

Gibbs, Buckner and Bloor, White and Rabinowitch. The atherosclerotic tendency among patients with diabetes, according to Duff, is not necessarily due to hypercholesteremia but may be a manifestation of other profound metabolic disturbances in this disease. Xanthomatosis, frequently accompanied by hypercholesteremia, often is associated with cardiac complications of probably arteriosclerotic origin, according to Thannhauser, Montgomery and Osterberg and C. Müller.

Before conclusions may be drawn regarding the influence of hypercholesteremia on the development of atherosclerosis in human beings, it is necessary to know the incidence of atherosclerosis in diseases accompanied by hypercholesteremia, such as hypothyroidism, nephrosis<sup>64</sup> and hepatic diseases. Opinions are contradictory. At present there is no convincing evidence that atherosclerosis results from hypercholesteremia alone. Apparently other factors are necessary. These are reviewed in an excellent summary of the chemical changes in atherosclerosis by Page.

#### DIABETES

Hyperlipemia is not now an important complication of diabetes. Before the era of insulin, hyperlipemia and hypercholesteremia were characteristic findings. Today, the adequately controlled diabetic patient may be expected to have normal blood lipid levels. An excellent review of the blood lipids in diabetes by Hunt shows that previous to 1924 the blood cholesterol in patients with diabetes averaged well over 300 mg. per hundred cubic centimeters; since 1930 the average has been practically normal: approximately 200 mg. per hundred cubic centimeters.

In cases of diabetic hyperlipemia, extreme values are often found for the blood lipids, concentrations of 20 (Hunt), 23 (Lichtenstein and Epstein) and 26 per cent (Klemperer) having been reported. According to Hunt, the cholesterol in milky, hyperlipemic serum varied from 75 to 1,600 mg. per hundred cubic centimeters. In fat content and appearance such serums resemble cream. The cholesterol, though raised to high levels, does not keep pace with the increase in neutral fat, which accounts for by far the greatest quantity of the lipids in hyperlipemic serum.

It should be emphasized that hypercholesteremia is not directly related to the severity of the diabetes. In a group of 43 cases with an average blood cholesterol value of 557 mg. per hundred cubic centimeters, for example, the average blood sugar was only 240 mg. per hundred cubic centimeters (Hunt). (It more probably is a result of

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64. A relationship between the serum cholesterol and atherosclerosis in chronic glomerulonephritis was shown in a recent article by Steiner and Domanski (*Am. J. M. Sc.* **204**:79, 1942).



lack of control.) As hypercholesteremia (or hyperlipemia) develops gradually in an uncontrolled diabetic patient and subsides slowly with treatment, the cholesterol level of the blood is a good index of the adequacy of treatment.

Hypercholesteremia in a case of diabetic coma (Hunt) is not very serious, and in this case the prognosis is no worse than in other cases of coma. If of short duration, the hypercholesteremia with adequate control subsides without permanent damage. If the hyperlipemia is longer lasting, the prognosis is bad because of the presence or imminence of serious complications. For instance, of 13 uncontrolled diabetic children studied carefully (Hunt), 12 exhibited serious complications, such as arteriosclerosis and xanthomatosis. The seriousness of hypercholesteremia in diabetes, apart from the fact that it signifies disorganization in the metabolism of carbohydrate, lies in the tendency for cholesterol when in high concentration in the serum to be deposited in the tissues, which gives rise to the various lipidoses, atherosclerosis, xanthoma diabeticorum and lipemia retinalis (Thannhauser).

The finding of a low level of blood cholesterol is, however, far more serious. Patients with values of 90 mg. per hundred cubic centimeters or less have a high mortality (Hunt). This condition, fortunately, is rare. It is accompanied in most instances by endocrine disorders and hepatic injuries unrelated to diabetes but to which the diabetes may be a contributing factor.

*Lipid Metabolism in Diabetes.*—Although acidosis and coma often are associated with high values for blood cholesterol, there is no relation between the height of the blood cholesterol and the degree of acidosis. Thus, in Joslin's clinic (Hunt), in 5 cases with an average carbon dioxide-combining power of 2 volumes per cent the average value for blood cholesterol was 321 mg., and in 14 cases with a carbon dioxide-combining power of 19 volumes per cent it was 311 mg. Man and Peters (1933) also reported the blood cholesterol in 15 cases of diabetic coma to be near or within normal limits. Though most of the studies of lipids in patients with diabetes, especially the clinical, emphasize the hypercholesteremia, the primary derangement is in the neutral fat fraction. This fraction exhibits the greatest fluctuation and is the cause of the milky appearance of the serum. The mechanism by which hyperlipemia is produced in the diabetic patient uncontrolled by insulin is unknown. The proper understanding of this problem undoubtedly must await fundamental chemical and physiologic studies of intermediary fat and carbohydrate metabolism. The complex relations between fats and sugars and their interconversion form one of the most important problems of the biochemist.

Whether or not insulin exerts a direct effect on the blood lipids is not known. In normal men, Bruger and Mosenthal found no con-

sistent effect of the administration of insulin on the blood cholesterol, whereas Randall observed a slight rise in schizophrenic patients treated with insulin. Decreases in blood cholesterol on the administration of insulin to animals were reported by Page, Pasternak and Burt (1931). As these effects were slight and variable, the beneficial effect of insulin on the hyperlipemia of diabetes may be secondary to and dependent on the establishment of normal carbohydrate metabolism.

#### XANTHOMATOSIS

The term "xanthomatosis" refers to diseases of obscure causation in which abnormal amounts of cholesterol are found in cells of the reticuloendothelial system. Because of its rarity, xanthomatosis may interest the physiologist and pathologist more than the clinician. The cutaneous, cerebral, cardiovascular and visceral symptoms of xanthomatosis presumably are merely local manifestations of similar underlying pathologic changes in the reticular cells. Thus the exophthalmos and the diabetes insipidus of the Schüller-Christian syndrome result from involvement of the dura and the brain; the jaundice associated with cutaneous xanthomatosis in some cases is a result of xanthomatous involvement of the liver or the bile ducts. Clinical, pathologic and chemical features of these diseases are reviewed by Montgomery and Osterberg, Thannhauser and Magendantz, and Thannhauser.

Thannhauser classified xanthomatous disease into primary essential xanthomatosis, due to an intracellular disorder of cholesterol metabolism, and secondary xanthomatosis due to hyperlipemia. Essential xanthomatosis may involve the skin, tendons, blood vessels, endocardium, bile ducts, bones and viscera. Secondary xanthomatous disease occurs only after long-standing hyperlipemia, as in uncontrolled diabetes (*xanthoma diabetorum*) or in idiopathic hyperlipemia. Cholesterol may be deposited in the liver, spleen, blood vessels and other organs, as well as in the skin.

The lesions of secondary xanthomatosis differ distinctly from those of the essential type. The characteristic foam cells of the latter are sparse in the former. Extracellular lipid deposits and inflammatory changes are absent in essential and present in secondary xanthomatosis.

The xanthomatous deposits, wherever located, exhibit in common a high lipid content, of which cholesterol is the major component. Eckstein and Wile analyzed a large nodule of *xanthoma tuberosum*, finding 17.6 per cent lipids, with cholesterol constituting 48.8 per cent of the total. Montgomery and Osterberg, in analyses of tissues in *xanthoma tuberosum*, reported values for total lipids ranging from 3.7 to 14.0 per cent, with cholesterol ranging from 18 to 64 per cent of the lipid fraction. By far the greatest amount of cholesterol is in the

combined form. Similar values were found in 2 cases of disseminate xanthomatosis. Thannhauser reported values of 5.5 and 1.31 per cent of the wet tissue for cholesterol in the cutaneous lesions of xanthoma tuberosum and xanthomatous biliary cirrhosis, respectively. Data for other lipids in xanthomas are too sparse for generalization.

According to Thannhauser, moderate increases in the blood phospholipid and fatty acids accompany xanthomatosis associated with hypercholesteremia. In 11 instances the serum cholesterol ranged from 210 to 667 mg. per hundred cubic centimeters. The increase was mainly in the ester fraction, except in xanthomatous biliary cirrhosis, in which the esters were low, probably because of hepatic damage.

The origin of the cholesterol in xanthomas is unknown. Though there is general agreement that the disease is a disorder of cholesterol metabolism, it is not clear whether the disturbance is in the blood, in some central organ, in the whole body or in the cells composing the lesion.

Hyperlipemia undoubtedly is often responsible for xanthoma formation. Xanthomatosis may occur with hyperlipemia of long standing, such as that in uncontrolled diabetes (xanthomatosis diabetorum) or in idiopathic familial hyperlipemia. The condition, designated appropriately by Thannhauser as secondary xanthomatosis, disappears if the blood lipids return to normal by dietary or other treatment. Strictly, it is not a disease but a manifestation of the hyperlipemia. Storage of lipids occurs presumably as a defensive mechanism of the reticulo-endothelial system for removing the excess lipids.

Other xanthomatoses, differing histologically from the secondary and designated "primary essential" by Thannhauser, may or may not be associated with high blood lipid levels. Xanthomatosis disseminatum and osseous and visceral xanthomatosis, including the Hand-Schüller-Christian syndrome, are not associated with any abnormalities in the blood lipids. Even in xanthomatoses of the skin (tuberosum and planum) and those involving tendons, blood vessels, the endocardium and bile ducts, usually accompanied by hyperlipemia, remission of the lesions does not depend on decreases in the blood lipids. The xanthomas, usually permanent, may disappear after a long time. Whatever the effect of the blood lipids in the genesis of essential xanthomatosis, it is not their amount alone that is important. In experimental xanthomatosis following cholesterol feeding in rabbits, Weinhouse and Hirsch found no relation between the level of the blood lipids and the amount of xanthomatous involvement of the blood vessels (atheroma) and skin.

The hypothesis suggested by Bloch and by Schaaf and Werner recognizes the importance of the lipid complex as a whole in the

evolution of the lipidoses. The blood lipids, insoluble in the ordinary sense, are held in colloidal suspension by a proper balance between the hydrophobic components, cholesterol and its esters and glycerides, and the hydrophilic phosphatides and possibly other unknown lipids, the whole complex being attached by loose physical or chemical attraction to the proteins of the serum. Any disturbance in the lipid balance or in the proportion of lipid to protein undoubtedly would lead to instability of the colloid, with consequent agglomeration into coarser particles. The avidity of reticulocytes and histiocytes for particulate matter is well known. The coarsely dispersed lipid complex is taken up by these cells, forming then the typical xanthoma cells. The objection to this theory is the absence of abnormality in the blood lipid picture in many cases of essential xanthomatosis.

The foregoing data indicate that although secondary xanthomatosis probably results from an increase in the quantity of cholesterol in the serum or from a disturbance in the colloidal state of the lipid complex, primary essential xanthomatosis may occur without obvious abnormality of the blood lipids.

Thannhauser considered essential xanthomatosis an intracellular disturbance of cholesterol metabolism, the disorder being localized in the xanthoma cells. This view regards the xanthoma cell as a metaplastic reticular cell or histiocyte, the transformation occurring as a result of the metabolic disturbance. The condition is analogous to Niemann-Pick or Gaucher's disease, in which sphingomyelin or cerebrocerebroside, respectively, is stored in the reticuloendothelial cells. Thannhauser designated essential xanthomatosis as metaplastic reticular and histiocytic cholesterosis, Gaucher's disease as metaplastic cerebrosidosis and Niemann-Pick disease as metaplastic sphingomyelinosis.

The nature of the metabolic disorder is unknown. Increase of lipid conceivably may result from increased synthesis within the cell or from impairment of the ability to break down these substances owing to the lack of a hormone or an enzyme. The transport of fatty acids among cholesterol, glycerides, phospholipids and cerebrocero- sides proceeds through exchange reactions, enzymatic in nature. The lack of one of these responsible for a single step in the series may result in the accumulation of one of the lipids. This hypothesis conforms with the often suggested role of the reticuloendothelial system in lipid metabolism. Because of the rarity of xanthomatosis and of reliable lipid analyses of blood and tissues in this disease, etiologic theories are necessarily speculative, and adequate understanding of the disease will have to await further knowledge of the functions of the reticuloendothelial system and its relations to lipid metabolism.

## INFECTIONS

Most investigators of the blood cholesterol in acute and chronic infections agree that its concentration increases in all febrile conditions and returns to normal during convalescence.<sup>55</sup> Boyd (1935 j) and Stoesser and McQuarrie found that the decrease was accompanied by comparable decreases in phospholipids. These changes were not dependent on diet or on changes in blood concentration. No constant relation was observed between the rise in the blood cholesterol and the height of the fever, the severity of clinical symptoms or the white cell count. Because of the well known tendency for cholesterol to combine with and detoxify saponins and other toxins, many authors have suggested that the lowering of the blood cholesterol is due to the combination of this lipid with toxins produced by the infectious process (Bills). This hypothesis refutes the facts disclosed by Ewert (1935), who observed in aseptic hyperthermia brought about by chemicals the same lowering as in infectious fevers. It is, furthermore, hard to understand how the extremely minute amounts of toxins circulating in the blood stream during infections could combine with and remove amounts of cholesterol sufficient to create demonstrable hypocholesteremia. The lipopenia of fever more probably is a result of increased metabolism, similar to the blood lipid picture in hyperthyroidism.

## TUMORS

Despite the known cancerigenic action of chemical substances closely related to cholesterol, there is no clear physiologic relation between cholesterol metabolism and tumor formation. That certain products of the breakdown of cholesterol in the body cause cancer is an intriguing and challenging hypothesis. To date, however, no evidence has been found of an abnormality in the blood lipids of cancerous persons. Evidence discussed by Roffo and by Knudson, Sturges and Bryan indicates that an increase in the cholesterol content of the skin predisposes to tumor formation in this region. This is apparently a local condition having no connection with the cholesterol content of the blood.

## MENTAL DISEASES

The relationship of cholesterol metabolism to mental disease is a well explored subject, but again no clear relation has been established. Cholesterol metabolism may be associated with mental processes, nerve tissues being rich in this substance. All opinions, however, must remain conjectural until fundamental biologic studies establish the role of cholesterol and other lipids in the structure and the function of nerve tissues.

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56. Boyd, 1935 j. Poindexter. Stoesser and McQuarrie.

## DISEASE OF THE ENDOCRINE GLANDS

The relationship of the endocrine glands, particularly of the gonads, to the lipid metabolism, is a fascinating subject, and the future undoubtedly will see tremendous advances in this field. At present, an orderly exposition of the relationship of lipids to this rapidly expanding field is difficult.

## IV. FUNCTION OF CHOLESTEROL IN THE BODY

The ubiquitous occurrence of cholesterol in the animal kingdom suggests that this substance takes part in fundamental metabolic processes. Despite the number of functions ascribed to cholesterol, no single one has been established with certainty. Bills's outline of the functions which at various times have been attributed to cholesterol will be reviewed briefly.

The chemical similarity between cholesterol and the bile acids, the adrenal hormones, estrogen and androgen and vitamin D suggests several possible functions, but no physiologic relation has yet been established. Cholesterol has been suggested as a "conditioner" of the skin, as an instrument for the conveyance of fatty acids in the body by exchange esterification with glycerides and phospholipids, as an insulator for the myelin sheath and as an important part of the structure of the cell membrane.

The fact that cholesterol has a neutralizing action against hemolytic substances, such as snake venoms, saponins and bacterial toxins, suggests an important function, but again there is no proof that cholesterol in the blood or the tissues serves such a purpose.

Regardless of the function of cholesterol in the body, it must be emphasized that its action probably is associated closely with that of the other lipids. As the previous pages have shown, any change in the blood cholesterol is accompanied invariably by a comparable one in the phospholipid and glyceride components. Before the changes in the blood cholesterol in the presence of disease may be understood, considerable study will have to be devoted to the functions and the metabolism of cholesterol and the relations of this to the other lipids.

Complete blood lipid analyses in cases of metabolic disease would be highly desirable; such determinations are long and tedious, however, and too complex for the average technician to master. Hence this type of investigation can be carried out at present only by expert chemists. The adoption of complete lipid analyses as a routine hospital laboratory procedure must await the discovery of better and simpler methods.

Reliable and simple methods for the determination of free and esterified cholesterol are available. Coupled with a knowledge of the

normal level and the changes to be expected under physiologic conditions, blood cholesterol values may contribute greatly in the diagnosis of disease.

One reason for contradictory reports is the frequent employment of so-called clinical methods of analysis, which are grossly inadequate with respect to completeness of extraction or analytic precision. A detailed criticism of each study is, however, far beyond the scope of this review. Doubtless better agreement will come with more general adoption of reliable analytic procedures. Another source of conflict is more apparent than real; that is, conclusions often have been based on observed differences within the order of magnitude of the experimental error of the method employed.

There is a strong probability, however, that the lack of agreement, so characteristic of data in the field of the blood lipids, may be caused by the lack of control of one or more hitherto unrecognized factors. For example, the effect of variations in the water content of the blood which occur under certain conditions hardly has been considered. Determinations of cholesterol paralleled by measurement of the specific gravity of the fluid possibly may yield a truer picture of the blood cholesterol than exists at present.

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## Notes and News

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**Appointments, Etc.**—Alan N. Drury, Huddersfield lecturer in special pathology at the University of Cambridge and a member of the scientific staff of the Medical Research Council, will become director of the Lister Institute, London, March 31, on the retirement of Sir John C. G. Ledingham.

William F. Petersen has resigned as professor of pathology in the University of Illinois College of Medicine.

**Awards.**—The Chicago Section of the American Chemical Society has awarded its thirty-second Willard Gibbs Medal to Conrad A. Elvehjem, professor of biochemistry in the University of Wisconsin, in recognition of his original contributions in biochemistry.

Leslie T. Webster, of the Rockefeller Institute for Medical Research, received the fifth annual award of the Dog Writers Association for his work on the diseases of dogs, especially rabies.

**Society News.**—The American Association of Pathologists and Bacteriologists and the American Association of Immunologists have decided to omit their 1943 meetings.

**Deaths.**—Leo Zon, passed assistant surgeon of the United States Public Health Service and pathologist to the United States Marine Hospital in Baltimore, has died.

Edgar Allen, professor of anatomy in Yale University, outstanding investigator in anatomy, physiology and endocrinology, died on February 3 at the age of 50 years.

**Children's Tumor Registry.**—Under the auspices of the American Academy of Pediatrics, a registry of tumors in childhood has been established in the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. The material will include all forms of cancer as well as "certain benign tumors" in children less than 15 years of age.

**Public Health Research Institute of the City of New York.**—This is said to be the first institute of its kind. It is a nonprofit institution for fundamental research in medicine, public health and other problems, authorized by the state legislature. O. A. Bessey, department of pathology at Harvard Medical School, becomes director in the place of Ralph Muchenfuss, who has entered war service.

**Bequest for Cancer Research.**—It is reported that about \$2,000,000 has been bequeathed to the Lankenau Hospital, Philadelphia, for cancer research.



## Books Received

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REPORT OF THE COMMITTEE ON TUBERCULOSIS IN WAR-TIME, SPECIAL REPORT SERIES No. 246. Privy Council Medical Research Council. Pp. 36. Price 9d. London: His Majesty's Stationery Office, 1942.

THE HEMORRHAGIC DISEASES AND THE PHYSIOLOGY OF HEMOSTASIS. Armand J. Quick, Ph.D., M.D., associate professor of pharmacology, Marquette University School of Medicine, Milwaukee, Wis. Vol. 8. Pages 340, with 23 figures. Price \$5. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

HUMAN EMBRYOLOGY. Joseph Krafka Jr., M.D., Ph.D., professor of microscopic anatomy, University of Georgia School of Medicine. Pp. 395, with 222 illustrations. Price \$4.75. New York and London: Paul B. Hoeber, Inc., 1942.

A concise textbook of human embryology designed to give the medical student the essentials of the subject in the time now allotted to it by the curriculum.

AUTONOMIC REGULATIONS: THEIR SIGNIFICANCE FOR PHYSIOLOGY, PSYCHOLOGY AND NEUROPSYCHIATRY. Ernst Gellhorn, M.D., Ph.D., professor of physiology, University of Illinois College of Medicine. Pp. 373, with 80 illustrations. Price \$5.50. New York: Interscience Publishers, Inc., 1943.

OVARIAN TUMORS. Samuel H. Geist, M.D., attending gynecologist, Mount Sinai Hospital; clinical professor of gynecology, College of Physicians and Surgeons, Columbia University. Pp. 527, with 266 figures. Price \$10.50. New York: Paul B. Hoeber, Inc., 1942.

FUNDAMENTALS OF IMMUNOLOGY. William C. Boyd, Ph.D., associate professor of biochemistry, Boston University School of Medicine; associate member, Evans Memorial, Massachusetts Memorial Hospitals, Boston. Pp. 446. Price \$5.50. New York: Interscience Publishers, Inc., 1943.

This is an excellent book for scientists and graduate students who are prepared to delve deeply into the science of immunology, but it is rather advanced and elaborate to serve as an introductory textbook for the undergraduate medical student. This statement applies particularly to the chapters on the basic principles of immunology.

FAMILIAL NONREAGINIC FOOD-ALLERGY. Arthur F. Coca, M.D., medical director, Lederle Laboratories. Pp. 160, with 20 tables and 12 charts. Price \$3. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

The author describes tachycardia as associated with morbid reactions to various kinds of food. Tachycardia may serve to identify the particular food it concerns in a given case.

## VISCERAL LESIONS ASSOCIATED WITH CHRONIC INFECTIOUS (RHEUMATOID) ARTHRITIS

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Patients suffering with rheumatoid arthritis frequently present much evidence that the disease is not confined to the joints. For this reason many physicians have felt that rheumatoid arthritis is a generalized disease of which the arthritis is but a single manifestation. From a clinical standpoint it is easy to detect profound disturbances of the functions of a number of organs among such patients but curiously, only fragmentary data concerning the visceral pathologic changes of this disease have been reported. Consequently, little is known as to the morphologic changes which may be at the basis of the visceral or systemic manifestations of rheumatoid arthritis.

In 1890 Garrod<sup>1</sup> wrote that such visceral lesions as may be found at necropsy in patients who have suffered from rheumatoid arthritis are, with a few possible exceptions, ascribable to intercurrent diseases and not to the disease of the joints. This view has been accepted almost without question to the present time and the same view was reflected only recently in a publication by a well known modern authority on arthritis (Copeman<sup>2</sup>), who stated that the viscera are "rarely" affected in rheumatoid arthritis and that the disease therefore rarely shortens life. Kuhns<sup>3</sup> and Kuhns and Joplin<sup>4</sup> have reported recently on 76 cases of "atrophic arthritis," in about half of which, according to a personal communication, necropsy was performed. They reported that in many of their cases arteriosclerosis had developed rapidly, and they noted that an unstated number of their patients had resulting myocardial

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From the Section on Pathologic Anatomy (Dr. Baggenstoss) and the Division of Medicine (Dr. Rosenberg) of the Mayo Clinic.

1. Garrod, A. E.: *A Treatise on Rheumatism and Rheumatoid Arthritis*, Philadelphia, P. Blakiston, Son & Co., 1890, pp. 261-263.

2. Copeman, W. S. C.: *J. Roy. Inst. Pub. Health & Hyg.* **1**:623, 1938.

3. Kuhns, J. G.: Personal communication to the authors.

4. Kuhns, J. G., and Joplin, R. J.: *New England J. Med.* **215**:268, 1936.

or renal lesions. Death was said to have been caused by pneumonia in 18, by myocarditis in 13, by nephritis in 11 and by postoperative complications in 6. In 28 instances, the causes of death were "miscellaneous." No more intimate description of the visceral lesions was reported in their paper.

Still's disease, which is considered to be a form of rheumatoid arthritis present in children, was described recently by Portis<sup>5</sup> as being associated with distinct, though not pathognomonic, visceral lesions. The findings included hyperplastic lymph nodes. Histologic examination revealed proliferation of reticulum cells in the lymph nodes, hyperplasia of the spleen, and passive congestion and fatty degeneration of the liver.

With the exception of these studies, no extensive reports of necropsies on the visceral aspects of rheumatoid arthritis have been made in recent years. We undertook the present study primarily with a view to determining the nature of the anatomic changes which occur in the viscera of these patients. We also hoped that we might be able to throw some light on the present obscure causation and genesis of this disease.

#### MATERIAL AND METHODS

We included in our study the necropsies in all cases of rheumatoid arthritis. We found that a total of 30 cases had accumulated to this time. These 30 cases include 25 which formed the basis of a previous investigation on cardiac lesions of patients who had had rheumatoid arthritis, and 5 additional cases in which necropsy had been carried out since that paper was published. Our series was chosen so that it would include every instance of rheumatoid arthritis in the necropsy material of the Mayo Clinic. Our only requirement for inclusion in this study was that acceptable clinical criteria for the diagnosis of rheumatoid arthritis must have been fulfilled and evidence of the presence of these features must have appeared in the records. Our criteria were outlined in previous papers and are not repeated here. In each case an internist particularly interested in arthritis and the rheumatic diseases had passed on the diagnosis of rheumatoid arthritis. We found that only 2 patients recalled an illness which might have been rheumatic fever.

The patients included 17 men and 13 women. The mean age of these patients at the time of death was 37.6 years. The youngest patient was 9 years and the oldest 81 years of age. Only 5 patients were 60 years of age or more, and 18 were less than 50 years of age. Eleven of the patients were less than 40 years of age.

The data used for this study were obtained by first abstracting carefully the available clinical records of these patients. Subsequently we made gross examinations of all organs and made appropriate sections for histologic examinations from all structures which presented an abnormal gross appearance. Routinely also sections were taken from the heart and its valves, the adrenal glands, the kidneys, the lungs, the spleen, the liver and the pancreas even though these organs appeared normal at the time of the gross examinations.

The sections were stained routinely with hematoxylin and eosin. Van Gieson, Mallory-Heidenhain and Gram stains and silver impregnation methods were employed when indicated.

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5. Portis, R. B.: *Am. J. Dis. Child.* 55:1000, 1938.

The causes of death in the cases studied are listed in table 1. It is necessary to point out, however, that necropsy frequently revealed serious lesions in more than one vital organ, so that an unequivocal decision as to the principal cause of the patient's death has not always been possible.

## OBSERVATIONS

*Cardiac Lesions.*—In a recent paper<sup>6</sup> we published details of the character of the lesions which we encountered in the hearts of 25 of the patients who constituted the present series. For the present we wish merely to summarize the findings in the hearts of the entire group.

In 16 (53 per cent) of the 30 patients we found rheumatic heart disease, and in 2 further patients we found lesions which may have been rheumatic in origin. The lesions of the 2 latter included calcific aortic stenosis and chronic pericarditis.

TABLE 1.—Cause of Death in Thirty Cases of Rheumatoid Arthritis

Cause of Death	Cases
Cardiac disease (9 cases)	
Rheumatic cardiac disease.....	7
Nonrheumatic cardiac disease.....	2
Renal disease (3 cases)	
Acute pyelonephritis with oliguria.....	2
Renal amyloidosis .....	1
Pulmonary disease (11 cases)	
Chronic bronchietasis with pulmonary suppuration.....	2
Pulmonary embolism .....	3
Bronchopneumonia .....	3
Pulmonary fat embolism.....	2
Postoperative massive collapse.....	1
Intestinal disease (2 cases)	
Chronic diarrhea of undetermined origin.....	2
Miscellaneous causes (5 cases)	
Cinchophen hepatitis .....	1
Violent accidental death.....	1
Carcinoma of prostate with metastasis.....	1
Sudden unexplained death.....	1
Cause of death unknown.....	1
Total .....	30

In these 2 instances, however, the rheumatic nature of the heart disease was not certain. It was our opinion that 7 of the 16 patients with rheumatic heart disease had met death as a direct consequence of that disease. In 2 others rheumatic heart disease was a contributory cause of death.

Two of our patients died as a result of nonrheumatic forms of heart disease: one of coronary arterial occlusion and the other of severe myocardial degeneration of an undetermined cause.

In 6 patients nonrheumatic cardiac lesions were present, but these were minor in severity and were not considered to have contributed significantly to the cause of death. These lesions included (1) coronary sclerosis with chronic myocardial infarction, (2) hypertrophy of the heart resulting from hypertension, (3) hydropericardium, (4) nonspecific subacute pericarditis, (5) chronic obliterative pericarditis and (6) calcific aortic stenosis.

The number of patients in this series who gave a history of rheumatic fever was small; only 2 recalled an illness the description of which fitted that condi-

6. Baggenstoss, A. H., and Rosenberg, E. F.: Arch. Int. Med. 67:241, 1941.

tion. This low number with a history of rheumatic fever is in striking contrast to the large number, 16, in whom we found clear evidence of rheumatic heart disease at necropsy.

Another notable observation of our study is the fact that clinicians had been able to discover clear evidence of rheumatic heart disease in only 1 of the 16 patients in whom that type of heart disease was present as proved by necropsy. This discrepancy is entirely unexplained as yet.

From a clinical standpoint it has long been known that the incidence of signs of rheumatic heart disease among patients suffering from rheumatoid arthritis is low. After encountering so large an incidence of rheumatic heart disease in the patients of this series, we restudied a number of patients suffering from rheumatoid arthritis in our wards to determine whether we had been overlooking minor signs of rheumatic heart disease. The result served only to confirm our previous clinical impressions, for we did not find a single instance of detectable

TABLE 2.—*Postmortem Observations on the Lungs in Thirty Cases of Rheumatoid Arthritis*

	Cases
Fibrous adhesions between parietal and visceral pleura (22 cases)	
Associated with pericardial adhesions.....	10
Associated with healed tuberculosis of lungs and hilar nodes.....	7
Associated with healed tuberculosis and hilar nodes only....	4
Associated with chronic active tuberculosis of lungs.....	3
Bronchopneumonia (9 cases)	
Responsible for death.....	3
Terminal .....	6
With empyema .....	2
With pulmonary abscess.....	1
With organization .....	2
Bronchiectasis (3 cases)	
Mild .....	1
Severe (responsible for death).....	2
Pulmonary embolism (4 cases)	
Fatal .....	3
Nonfatal .....	1
Pulmonary fat embolism.....	2
Emphysema .....	3
Bronchitis .....	1

rheumatic heart disease in a service of 15 persons suffering from rheumatoid arthritis.

Thus our findings in the hearts of patients in this series raise questions which cannot be answered satisfactorily at the present time: the question whether rheumatoid arthritis is responsible for a form of heart disease indistinguishable from rheumatic heart disease, and the question whether rheumatoid arthritis and rheumatic fever are related.

*Pulmonary Lesions.*—The notable pulmonary lesions encountered among these patients are outlined in table 2. Although we searched with care, we failed to find pulmonary lesions which might in any way be considered specific for rheumatoid arthritis. We likewise did not observe any condition which might be labeled rheumatic pneumonia despite the high incidence of rheumatic heart disease among these patients.

Fibrous adhesions, indicating episodes of pleurisy with healing, were noted in 22 cases. Since pleural adhesions are a common manifestation of tuberculosis, the possibility that these adhesions were the result of tuberculosis was considered. In a careful search for evidence of pulmonary tuberculosis, healed or chronic

tuberculous lesions were found elsewhere in the lungs in only 14 of the 22 cases in which there was healed pleuritis. We cannot be certain as to the relation of these evidences of healed pleuritis to rheumatoid arthritis, but we must assume that the inflammatory process of rheumatoid arthritis may manifest itself in the pleura as well as in the serous membranes of joints.

The presence of bronchopneumonia in 9 of the patients does not appear to be an unusually high incidence for a series of necropsies. It is noteworthy, however, that fatal bronchopneumonia was precipitated three times by various therapeutic measures directed against the arthritis, including typhoid vaccine shock therapy, tonsillectomy and lumbar sympathectomy.

TABLE 3.—*Postmortem Observations on the Liver in Thirty Cases of Rheumatoid Arthritis*

	Cases
Mean weight (23 cases only) 1,634.6 Gm.	
Heaviest (2,765 Gm.)	
Lightest (1,046 Gm.)	
Weighing more than 1,800 Gm.....	8
Weighing more than 2,000 Gm.....	4
Weighing less than 1,600 Gm.....	10
Weighing less than 1,400 Gm.....	8
Weighing less than 1,200 Gm.....	4
Chronic passive congestion with atrophy of cells about central veins (18 cases)	
Grade 1* .....	8
Grade 2 .....	9
Grade 3 .....	1
Central necrosis (4 cases)	
Cause of death; rheumatic heart disease.....	2
Cause of death; pulmonary infections .....	2
Fatty change (7 cases)	
Grade 1* .....	2
Grade 2 .....	4
Grade 3 .....	1
Amyloid deposits .....	1
Subacute yellow atrophy (einekophen hepatitis).....	1
Healed milary tuberculosis.....	1
Serous hepatitis (Rössle and Eppinger <sup>9</sup> ).....	9

\* The change was graded on the basis of 1 to 4, in which 1 designates the mildest and 4 the most severe condition.

The incidence of bronchiectasis, which was present in 3 patients, was not striking, and the lesions of this condition in our patients were in no way distinctive.

The incidence of emphysema in 3 patients and of bronchitis in 1 appeared to have been purely coincidental.

The occurrence of fat embolism in patients of this series was to be expected, for the literature contains a number of instances of death from this cause among rheumatoid patients.<sup>7</sup> A common accompaniment of rheumatoid arthritis is epiphyseal osteoporosis, a condition which increases the likelihood of fat embolism, because fracture of the osteoporotic bone may release significant amounts of fat into the venous circulation. Such fractures may be incurred even during careful orthopedic or physical therapy manipulations. Such a train of circumstances probably took place in the 2 fatal instances in our series.

*Hepatic Lesions.*—The notable observations relating to the liver are outlined in table 3. A curious healing effect of hepatic injuries on rheumatoid arthritis

7. Rosenberg, E. F.; Baggenstoss, A. H., and Hench, P. S.: Unpublished data.

and fibrositis has been described by Hench.<sup>8</sup> We have not, however, found any hepatic lesions which could be considered specific for this disease.

Hypertrophy of the liver was moderately frequent, as indicated by the fact that the weight exceeded 1,800 Gm. in 8 adults. It was the result of chronic passive congestion in some and of fatty change in others. We did not encounter any evidence to suggest that the reticuloendothelial system was stimulated to proliferate in the liver as it is at times in the spleen and the lymph nodes of patients suffering from rheumatoid arthritis.

Ten livers weighed less than 1,600 Gm., indicating that atrophy of the liver was even more common than hypertrophy. It was associated with inanition and general visceral atrophy in 7 cases but was present without associated wasting of the body in 3 cases. In 6 of the 10 cases in which there was hepatic atrophy there was also chronic passive congestion of the liver, and we believe that the atrophy was caused by this long-standing chronic passive congestion.

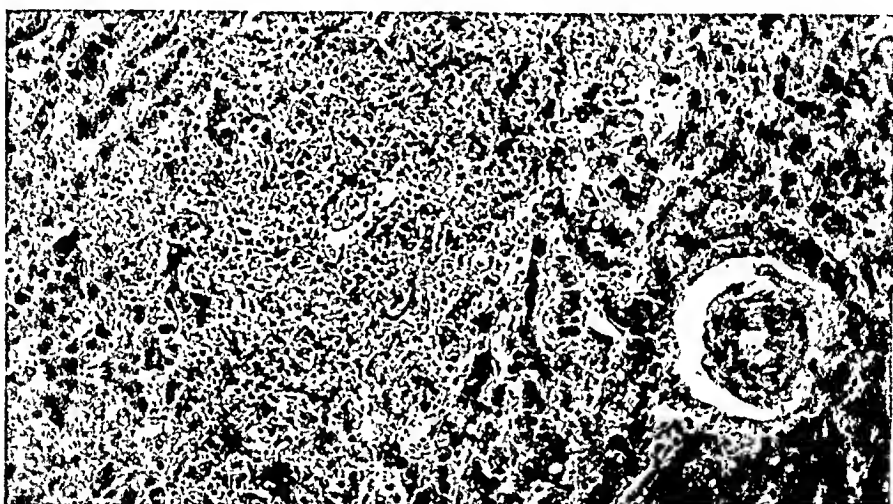


Fig. 1.—Liver. Mild chronic passive congestion with necrosis of cells about a central vein. So-called toxic central necrosis. Hematoxylin and eosin;  $\times 120$ .

The incidence of chronic passive congestion of the liver was high, this condition being present in 18 cases. It reflected the high incidence of serious heart disease. The histologic preparations of the livers in these cases always disclosed atrophy of the parenchymal cells about the central veins. This atrophy had doubtlessly resulted from the passive congestion.

A significant degree of hepatic central necrosis (fig. 1) affecting parenchymal cells was observed in 4 instances. This central necrosis was always associated with rheumatic heart disease and with severe and extremely crippling rheumatoid arthritis. We have no definite information as to whether any of the 4 patients had taken drugs containing cinchophen. The deaths of 2 had been caused by rheumatic heart disease, the death of a third by bronchopneumonia and that of the fourth by bronchiectasis and pulmonary suppuration. None of these factors alone explains satisfactorily the presence of hepatic necrosis, but we must admit the possibility that damage to the liver might have been caused by these diseases or by some remedy which may have been taken for the rheumatism.

Some degree of hepatic fatty change was observed in 7 cases. As we reviewed our data in search of an explanation we found that this lesion was associated

8. Hench, P. S.: *M. Clin. North America* 24:1209, 1940.

with an acute infection in 5 cases: bronchopneumonia in 2, pulmonary suppuration and bronchitis in 1, acute pyelonephritis in 1 and abscesses in the prostate in 1. In 3 further cases hepatic fatty change was associated with extremely severe clinical forms of rheumatoid arthritis as well as with rheumatic heart disease. In 2 cases, however, fatty change was associated with only moderate grades of rheumatoid arthritis and with no other serious visceral lesions. We therefore considered that fatty change might have reflected only the poor nutritional status of the patients during the days immediately preceding death, but it is possible that fatty change in the liver has a more direct significance in relation to rheumatoid arthritis.

Lesions which we identified as "serous hepatitis"<sup>9</sup> were observed in 9 cases. Usually we found this associated with some degree of passive congestion and atrophy of liver cells. Histologically, serous hepatitis is a separation of the reticulum fibers from the liver cells. Its significance is unknown. It may possibly result, as an artefact, from shrinkage of tissues during fixation and dehydration and thus may not have pathologic significance. We could not correlate this lesion with the severity or with the duration of the arthritis.

TABLE 4.—*Postmortem Observations on the Lymph Nodes in Seventeen Cases of Rheumatoid Arthritis*

	Cases
Proliferation of reticuloendothelial cells in sinuses.....	7
Proliferation of reticuloendothelial cells in follicles.....	1
Suppurative lymphadenitis (2 cases)	
Left inguinal nodes.....	1
Peripancreatic nodes .....	1
Degeneration of secondary centers of lymph follicles.....	2
Amyloid deposits .....	2

*Lymph Nodes.*—The lymph nodes, like other viscera, failed to reveal any lesion which could be considered pathognomonic of rheumatoid arthritis. Our findings as a result of a study of the lymph nodes in 17 of the cases are summarized in table 4.

Our series does not include any cases in which striking generalized enlargement of lymph nodes had been present during life. Moderate hypertrophy of lymph nodes such as we encountered has long been a recognized accompaniment of rheumatoid arthritis. Generally it is found in nodes which drain regions where there are affected joints, but occasionally all nodes are enlarged, and in some instances the spleen and the liver are also clinically enlarged. Notable descriptions of the association of enlargement of the spleen, the liver and lymph nodes with rheumatoid arthritis are those of Chauffard and Ramond,<sup>10</sup> Herringham,<sup>11</sup> Still,<sup>12</sup> Kauffman,<sup>13</sup> Collins,<sup>14</sup> Felty<sup>15</sup> and Hanrahan and Miller.<sup>16</sup>

9. Rössle and Eppinger, cited by Klemperer, P., and Keschner, H. W.: *Am. J. Path.* **12**:797, 1936.

10. Chauffard, A., and Ramond, F.: *Rev. de méd., Paris* **16**:345, 1896.

11. Herringham, W. P.: *Clin. J.* **34**:257, 1909.

12. Still, G. F.: *Med.-Chir. Tr., London* **80**:47, 1897.

13. Kauffman, D. E.: *J. Missouri M. A.* **34**:157, 1937.

14. Collins, D. H.: *Rep. Chron. Rheumat. Dis.* **3**:49, 1937.

15. Felty, A. R.: *Bull. Johns Hopkins Hosp.* **35**:16, 1924.

16. Hanrahan, E. M., Jr., and Miller, S. R.: *J. A. M. A.* **99**:1247, 1932.



The histologic observations in our own cases were in essential agreement with those noted in previous descriptions. The lesion which we noted most commonly and which has been reported repeatedly in the past was hyperplasia of the reticuloendothelial cells both within sinuses and within follicles.

Suppurative lymphadenitis of the inguinal nodes was noted in 1 case of cellulitis of the leg, and suppurative lymphadenitis of the peripancreatic nodes was found in another case in which we could not find any cause for this lesion.

Cellular degeneration of secondary centers of lymph follicles was noted in 2 cases. This condition is of uncertain cause. Amyloid deposits were present in 2 cases.

*The Spleen.*—Notable observations regarding the size of the spleen and the lesions present are assembled in table 5. Our studies of the spleens have failed to reveal any pathognomonic lesions characteristic of rheumatoid arthritis. We have noted that a significant enlargement of this organ was present in approximately 58 per cent of the patients (weight more than 200 Gm. in 14 of the 24 cases in which the weight of the spleen was known). This increased splenic weight was apparently caused by chronic passive congestion in 6 cases, and by

TABLE 5.—*Postmortem Observations on the Spleen in Thirty Cases of Rheumatoid Arthritis*

	Cases
Mean weight (24 cases) .....	250 Gm.
Lightest .....	86 Gm.
Heaviest .....	573 Gm.
Weighing 150-199 Gm. ....	6
Weighing 200-299 Gm. ....	5
Weighing 300 Gm. and more.....	9
Chronic passive congestion.....	8
Reticuloendothelial hyperplasia .....	5
With atrophy of malpighian corpuseles.....	2

reticuloendothelial hyperplasia in 5. We were interested to note, however, that histologic studies of 3 large spleens (323, 335 and 345 Gm.) did not show anything of note. The greatest weight, 573 Gm., was encountered in the spleen of a patient who had had severely deforming rheumatoid arthritis and whose death had resulted from pulmonary fat embolism. The outstanding histologic abnormality of this spleen was proliferation of the reticuloendothelial cells.

The incidence of chronic passive congestion of the spleen was high (present in 8 cases, or 27 per cent), but one might compare this incidence to that in the liver, for in both a high incidence of chronic passive congestion could be directly related to severe heart disease and congestive failure.

Degenerative changes within the bodies of the malpighian corpuscles were noted in 5 cases and similar degenerative changes in secondary centers of the lymph nodes were noted in 2 instances. These changes have a nonspecific character which pathologists encounter in a great variety of circumstances and which we believe cannot, with present knowledge, be related specifically to rheumatoid arthritis.

In 2 cases of this series amyloid degeneration of the spleen and other organs was found to be associated with the rheumatoid arthritis. One of the patients, a man of 48 years, who had had rheumatoid arthritis of many joints for six years, also had amyloid disease involving the spleen, liver, kidneys, adrenal glands, esophagus, small intestine, bladder, lymph nodes and thymus. Associated lesions

were chronic rheumatic mitral endocarditis, chronic rheumatic aortitis and focal myocarditis, ascites (300 cc.) and bilateral hydrothorax (500 cc.). The other patient, a boy of 17 years, who had had rheumatoid arthritis for seven years, had amyloidosis of the spleen, kidneys and lymph nodes. Associated lesions were subacute rheumatic pericarditis and focal myocarditis, cardiac hypertrophy (425 Gm.—normal 225 Gm.) with dilatation of the left ventricle and fatty change of the myocardium, subacute duodenal ulcer, bilateral hydrothorax, emaciation of grade 3 and chronic suppurative prostatitis.

*Kidneys.*—Notable observations regarding the kidneys of the patients included in this series are assembled in table 6. In the kidneys, as in most of the other organs which we examined, we failed to find any specific lesion which could be designated as characteristic of rheumatoid arthritis. However, we were impressed by a high incidence (19 cases) of glomerular endothelial proliferation (fig. 2 *A* and *B*), a lesion which Bell<sup>17</sup> designated a form of glomerulitis. The clinical studies in these cases revealed that frequently the patients excreted urine containing light or, rarely, heavy traces of albumin. However, severe renal insufficiency did

TABLE 6.—*Postmortem Observations on the Kidneys in Thirty Cases of Rheumatoid Arthritis*

	Cases
Glomerulitis (19 cases)	
Grade 1 .....	13
Grade 2 .....	6
Chronic or subacute interstitial nephritis (pyelonephritis) (4 cases)	
Subacute suppurative pyelonephritis.....	3
Chronic nonsuppurative pyelonephritis.....	1
Amyloid degeneration .....	2
Nephrolithiasis with acute pyelitis.....	1
Dissecting aneurysm, right renal artery.....	1

not result in any of these instances. We suspect, therefore, that the glomerulitis was responsible for the traces of albumin in the urine.

Bell, whose studies of this glomerular lesion have been exhaustive, expressed the belief that it may result from irritation of glomerular capillaries by a variety of toxic substances, especially those derived from streptococci. With this opinion in mind it is of interest to note that among our 19 cases showing this lesion the infectious processes present were as follows: terminal bronchopneumonia in 6, chronic pulmonary suppuration in 3 and active or healed rheumatic endocarditis in 9. Since Bell found that only 18.8 per cent of patients dying of pneumonia had glomerulitis, one would not expect that the terminal bronchopneumonia present in our patients would result in a high incidence of glomerulitis. Moreover, 3 patients in whom we found this form of glomerular inflammation had neither suppurative foci nor any evidence of rheumatic heart disease. These considerations led us to suspect that the agent responsible for rheumatoid arthritis may also be responsible for low grade, essentially subclinical glomerulitis.

A notable association between typhoid vaccine shock therapy and fatal diffuse severe pyelonephritis associated with anuria was seen in 2 instances. (fig. 2 *C*). Neither of the 2 patients had shown any evidence of pyelonephritis before the typhoid vaccine was administered, and we have no satisfactory explanation for this

17. Bell, E. T.: *Am. J. Path.* 12:801, 1936.

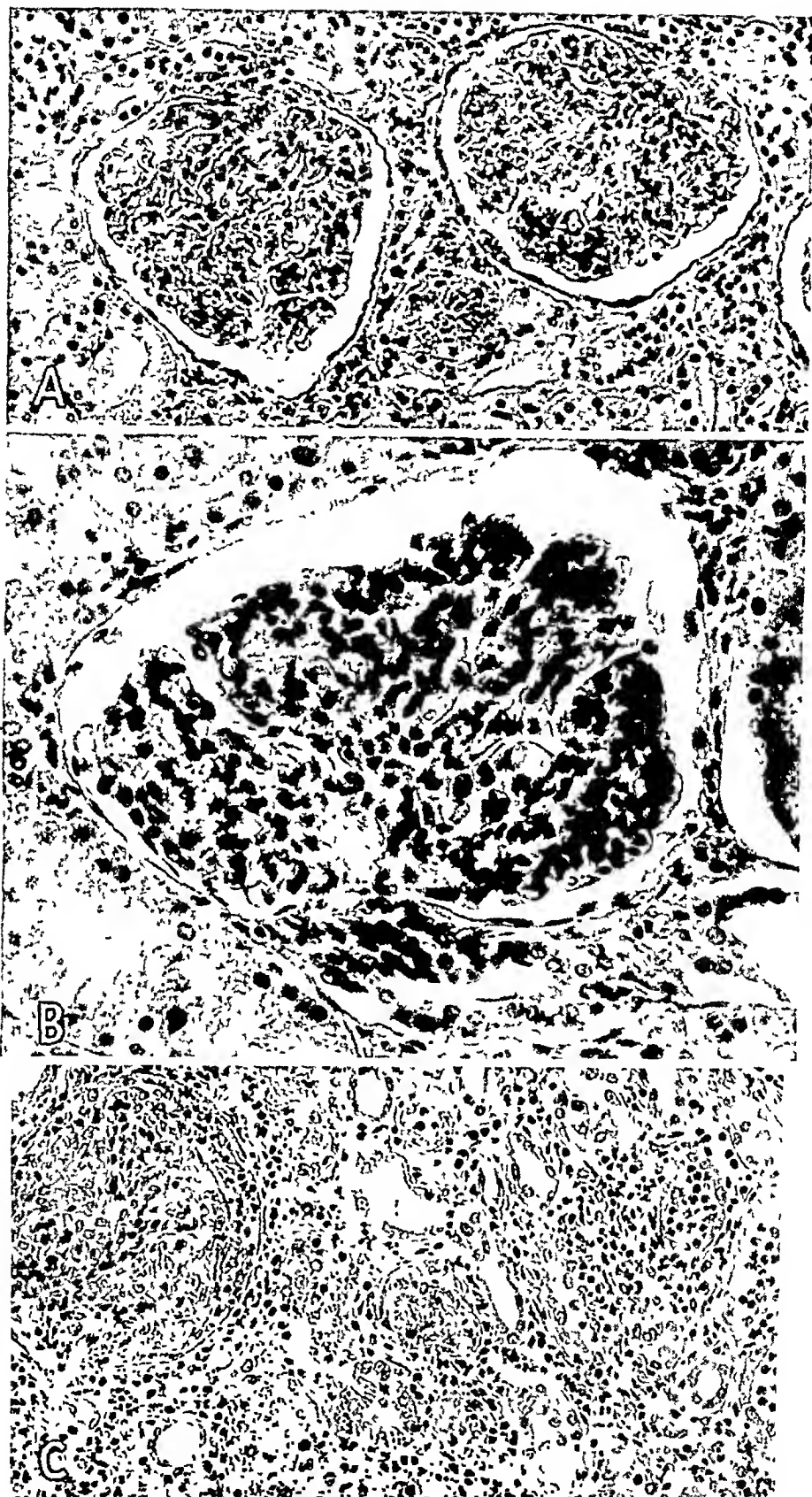


Figure 2

(See legend on opposite page)

curious untoward reaction to shock therapy. We consider this lesion to be a serious but fortunately very rare complication of treatment with typhoid vaccine.

There were 2 cases in which the glomeruli of the kidneys were the seat of amyloid deposits.

Other renal lesions are of only passing interest and represented probably only accidental associations with the rheumatoid arthritis. These included the following: dissecting aneurysm of the right renal artery which led to thrombosis of the artery and infarction of the kidney (1 case) and nephrolithiasis, associated with acute pyelitis (1 case).

*Gallbladder.*—Lesions found in the gallbladder included chronic cholecystitis in 2 cases (associated with stones in 1 case), uncomplicated cholelithiasis and acute purulent cholecystitis, each in 1 case. No relation of these lesions to rheumatoid arthritis was demonstrated.

*Gastrointestinal Tract.*—Various lesions were observed in the gastrointestinal tract, but for the most part these did not have any bearing on the arthritis. One patient who had generalized amyloidosis was found to have some amyloid deposited in the gastrointestinal tract. Other notable gastrointestinal lesions included ulcerative esophagitis (3 instances) and chronic ulcer, acute erosion and polypoid carcinoma of the stomach (1 instance each). Scars of healed duodenal ulcers were detected in 2 cases. A localized region of chronic inflammation in the small intestine was present in 1 instance. A few acute ulcers were observed in the colon of 1 patient and mild colitis without ulceration was seen in another.

*Pancreas.*—No pancreatic lesions were encountered which could be related in any significant way to rheumatoid arthritis. In 3 instances we found moderate degrees of chronic interstitial pancreatitis. In 1 of these 3 cases we encountered fat necrosis. The origin of these lesions is obscure; they are found in association with many other conditions. A moderate amount of fatty replacement was found in 3 additional cases.

*Adrenal Glands.*—The adrenal glands also failed to show any lesions which we could designate as a specific result of the rheumatoid arthritis. In 3 cases we found chronic passive congestion and considered this to be an accompaniment of severe heart disease. Focal collections of lymphocytes were encountered in the cortex in 1 case (a lesion fairly commonly present in occasional necropsies on persons who have died of varying causes). Amyloid deposits, atrophy, hemorrhage and periadrenal arteritis were observed each in 1 instance. We considered these lesions to be coincidental.

*Thyroid Gland.*—As one might expect, we encountered adenoma of the thyroid gland in a few (4) of the patients. Colloid goiter was noted in 4, and mild parenchymal hyperplasia was observed in 2. These lesions too were doubtlessly coincidental.

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#### EXPLANATION OF FIGURE 2

A, kidney. Endothelial proliferation (glomerulitis) grade 1 and hyaline granular degeneration of convoluted tubules. Hematoxylin and eosin;  $\times 205$ .

B, kidney. Glomerulitis grade 2. The capillaries are filled by a pronounced proliferation of endothelial cells. Hematoxylin and eosin;  $\times 350$ .

C, kidney. Suppurative interstitial nephritis (pyelonephritis) with involvement of a glomerulus. Hematoxylin and eosin;  $\times 190$ .

*Prostate.*—Lesions encountered in the prostate glands of the male patients in this series were considered by us to be coincidental and not directly related to the rheumatoid arthritis. Chronic suppurative prostatitis was found in 2 cases, and in 1 we encountered carcinoma.

*Blood Vessels.*—We examined the visceral blood vessels of these subjects with particular care because many of the clinical phenomena of rheumatoid arthritis indicate abnormal functioning of the vascular system. Thus, many of the patients suffer with tachycardia, cyanosis, abnormal sweating and discolorations of the extremities like those seen in Raynaud's disease. Despite these symptoms and despite the fact that we paid particular attention to the question of lesions in blood vessels, we have little to report in the way of significant findings in that system. As one might expect, in this group of relatively young persons we found arteriosclerosis to be of only moderate degree in general. In 20 of the patients the arteriosclerosis of the aorta was graded 1 (on the basis of 1 to 4); in 9 it was graded 2, and in only 1 case was it graded 4.

There was no widespread characteristic change among the smaller arteries or arterioles, and we did not find any significant lesions in the venous system which might be designated as a phenomenon of the rheumatoid arthritis.

#### COMMENT

The outstanding feature of the postmortem examinations of these patients was the finding of a high incidence of rheumatic heart disease. We have commented extensively on the finding in previous papers, and we believe it significant that among 5 patients who have been added to this series since the foregoing report was published we found certain rheumatic heart disease in 2 and a cardiac lesion which was possibly rheumatic in origin in 1. We have been interested to note that the publication of our paper was followed quickly by confirmatory reports of other postmortem studies in which a high incidence of rheumatic heart disease was found in patients suffering from rheumatoid arthritis.<sup>18</sup> This accumulating evidence of the high coincidence of the two conditions is becoming more and more impressive and suggests ever more strongly that rheumatoid arthritis and rheumatic fever are in some manner closely related conditions. The results of our study again focus attention on the researches of Klinge,<sup>19</sup> who concluded that rheumatoid arthritis and rheumatic fever are different manifestations of the same disease.

Second only in importance to the observations which we have reported concerning the hearts is the finding among the kidneys of these patients of a strikingly uniform and frequently present glomerulitis. This lesion was not extensive but it occurred with such frequency that we have had to conclude it is in some manner a sequel of, or accompaniment of, the disease of the joints. We considered the possibility that

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18. Andrus, F. C.: *Minnesota Med.* **24**:1071, 1941. Fingeraman, in discussion on Andrus, F. C.: *ibid.* **24**:1072, 1941. Dawson, H., and Bennett, G. A.: Unpublished data.

19. Klinge, F.: *Jahresk. f. ärztl. Fortbild.* **24**:1, 1933.

the glomerulitis might have resulted from the effects of other toxic or infectious states, and we feel certain that in some of the cases chronic pulmonary suppuration, and in others rheumatic infections, were responsible. We found the glomerulitis in some cases, however, without other discernible toxic or infectious process than rheumatoid arthritis.

Ever since the latter years of the nineteenth century, when Still<sup>12</sup> and Chauffard and Ramond<sup>10</sup> described the association of splenic and lymph node enlargements in cases of rheumatoid arthritis, pathologists and clinicians have been keenly interested in the changes which appear in these organs among arthritic patients. The consensus has been that no pathognomonic lesions appear in these organs, and our material also failed to disclose any characteristic lesions in the lymphatic system. Although some enlargement of the spleen was encountered commonly, we were forced to attribute this enlargement in most instances either to chronic passive congestion or to nonspecific proliferation of the reticulo-endothelial tissues.

With few exceptions, the anatomic changes which we encountered in the liver did not appear of sufficient gravity to have affected the known or clinically measurable functions of the liver. Chronic passive congestion and fatty change were the lesions most commonly encountered.

The results of our anatomic investigations have not led us to any final conclusions regarding the causation of rheumatoid arthritis. In general, the frequent evidence of inflammatory processes, such as we found in the hearts and the kidneys, together with the hyperplasia of the spleen and lymph nodes, favors the hypothesis that rheumatoid arthritis must be caused by some low grade infective agent. The presence of amyloidosis in 2 cases and the occasional presence of inflammatory lesions in the intestinal tract, the pancreas, the adrenal bodies and the prostate and even in the lungs might also be considered to favor that view.

Our data support the conception of rheumatoid arthritis as a generalized disease affecting not only the joints but also many viscera. However, we have been disappointed in general to find it necessary to arrive at the conclusion that our study has not cleared in any discernible manner the mystery surrounding the cause of the disease.

#### SUMMARY

Clinical records and necropsy data on 30 patients suffering from rheumatoid arthritis have been reviewed with a view to determining the nature of the visceral lesions in patients suffering with this disease.

Observations on the heart indicated that rheumatic cardiac disease was present in 16 patients (53 per cent) and cardiac lesions other than rheumatic in 8 (27 per cent).

The pulmonary diseases present among these patients included, notably, bronchopneumonia, fibrous pleuritis, bronchiectasis, pulmonary embolism and fat embolism. The interrelation of these pulmonary diseases with the rheumatoid arthritis has been reviewed.

Study of the liver failed to reveal any characteristic lesion which we could ascribe to rheumatoid arthritis. A number of abnormalities noted included hypertrophy, atrophy, chronic passive congestion, fatty change, central necrosis, amyloid deposits, subacute yellow atrophy, healed miliary tuberculosis and the serous hepatitis of Rössle and Eppinger.<sup>9</sup>

The lymph nodes and the spleen, though occasionally enlarged during life, were found to show only various nonspecific inflammatory and degenerative effects. These included proliferation of reticuloendothelial tissues, degeneration of lymph follicles, amyloid deposits, suppurative lymphadenitis, hypertrophy (of the spleen) and chronic passive congestion.

A striking result of this study was the finding of low grade nonspecific glomerulonephritis in 19 of our cases. The implications of this lesion have been considered. Other renal lesions present among these patients included chronic or subacute interstitial nephritis, nonsuppurative pyelonephritis, amyloid degeneration, nephrolithiasis with acute pyelitis and dissecting aneurysm of the right renal artery.

Various lesions, mainly of minor importance, were encountered in the other organs. These have been reviewed and catalogued.

# THE SHWARTZMAN PHENOMENON IN THE GENESIS OF PULMONARY ABSCESS

EXPERIMENTAL PRODUCTION OF ABSCESES IN THE LUNGS OF  
RABBITS BY MEANS OF A STRAIN OF GRAM-NEGATIVE  
ANAEROBIC BACILLI, RESEMBLING *BACILLUS NECROPH-*  
*ORUS*, EMPLOYED AS A LUNG-PREPARATORY  
FACTOR, WITH NOTES ON SOME  
FACTORS CONCERNED IN  
PATHOGENICITY

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In 1932 one of us (John Cohen †) published studies of putrid abscess of the lung, attempting to define the bacterial factors in its genesis.<sup>1</sup> In 16 cases of putrid pulmonary abscess he found a mixture of anaerobes of various types, among which were the following; a nonhemolytic "micro-aerophilic" streptococcus (*Streptococcus γ*) and a pleomorphic anaerobic diphtheroid, which were cultured from the pus in all 16 cases; *Bacterium melaninogenicum*, found in 13 cases; *B. ramosus*, in 8; fusiform bacilli, in 6; *Bacillus fragilis*, in 4; *Bacillus thetoides*, in 2; *Bacillus furcosus*, in 2; *Leptothrix*, in 3; *Vibrio*, in 1; *Clostridium cochlearium*, in 1. Aerobes were infrequently grown: *Streptococcus viridans* in 2 cases; Friedländer's bacillus in 1. He mentioned the etiologic importance of bacterial synergism in pulmonary abscess. This possibility had, in fact, been under discussion since the publication of the early researches of Guillemot, Hallé and Rist, in 1904.<sup>2</sup> He also pointed out the favoring effect on the in vitro growth of *B. melaninogenicum* of symbiotic cultivation with *Str. γ*.

Cohen's subsequent studies led to his interest in the Shwartzman phenomenon as the underlying mechanism in the production of pulmonary abscess by these micro-organisms. The Shwartzman phenomenon<sup>3a</sup>

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† Dr. Cohen died on Jan. 24, 1936.

From the Laboratories of the Mount Sinai Hospital.

1. Cohen, J.: Arch. Surg. **24**:171, 1932.

2. Guillemot, L.; Hallé, J., and Rist, E.: Arch. de méd. expér. et d'anat. path. **16**:571, 1904.

3. Shwartzman, G.: Phenomenon of Local Tissue Reactivity, and Its Immunological, Pathological and Clinical Significance, New York, Paul B. Hoeber, Inc., 1937, (a) chap. 1, p. 1; (b) chap. 7, p. 186; (c) chap. 8, p. 222.



consists in severe hemorrhagic necrosis rapidly evoked in a tissue at the site of a previous parenteral introduction of one or another of certain bacterial filtrates (preparatory factor) following an intravenous injection, made eighteen to twenty-four hours afterward, of a toxin (provided it is potent) consisting of a filtrate of a culture of the same or of another bacterium (provocative factor). The conventional tissue for its elicitation is the rabbit's skin. It may be produced in the kidneys, the gastric mucosa, the appendix, the synovia of joints, certain tumors (without previous preparation), the adrenal gland, the pancreas, lymph nodes and the other tissues except the brain and the meninges.<sup>3b, c</sup> It was produced in the lung in 1929 by Shwartzman employing *Bacillus typhosus* filtrate intratracheally as the preparatory factor.<sup>3b</sup>

Cohen<sup>4</sup> attempted to produce a potent toxin by the use of filtrates from cultures of anaerobes isolated from putrid pulmonary abscesses. He described experiments in which each of these filtrates was inoculated intradermally as the skin-preparatory factor and subsequently intravenously as the provocative factor. Negative results were obtained with filtrates of pure cultures of these anaerobes. When a potent heterologous toxin (e. g., *B. typhosus* or meningococcus toxin) was substituted as the skin-preparatory factor, the anaerobic toxin elicited a high incidence (80 per cent) of positive cutaneous reactions when given intravenously, indicating its potency at least as a provocative factor. On the other hand, strong skin-preparatory potency was found in filtrates of cultures of *B. melaninogenicum* and *Streptococcus γ* grown in symbiosis (9 positive results in 22 instances).

Some indication of the nature of the elements concerned in bacterial pathogenicity is possibly suggested by these differences in skin-preparatory potency of bacterial filtrates. As stated by Cohen,<sup>4</sup> "Further work on the pathogenicity of the various organisms, to be reported, points to the interesting fact that only those organisms which give the positive Shwartzman phenomenon when they are grown in suitable culture mediums are also able to produce severe necrotizing lesions in the lungs of rabbits."

In subsequent studies (unpublished) Cohen studied the toxin production of several other anaerobic micro-organisms from the pus of human pulmonary abscess. In the case of one of these (referred to in his notes as no. 7) he demonstrated the truth of his assumption, i. e., that its capacity in pure culture to produce a potent skin-preparatory toxin can be correlated with its ability to produce severe necrotizing lesions in the lungs of rabbits. The present report deals with an application of the Shwartzman phenomenon in the study of the pathogenicity of this anaerobe in the production of pulmonary abscesses in rabbits.

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4. Cohen, J.: J. Infect. Dis. 52:185, 1935.

## DESCRIPTION OF THE MICRO-ORGANISM

The anaerobic bacillus employed was isolated by Cohen from a human case of putrid abscess of the lung. For the details of its isolation and cultivation, the reader is referred to previous papers.<sup>5</sup>

*Cultural Characteristics.*—The micro-organism was one of a group of non-chromogenic, gram-negative, strictly anaerobic bacilli producing a foul odor in cultures. It varied in size in different mediums, appearing as coccoid or as slender straight or slightly curved bacillary forms 1 to 6 microns in length, with pronounced polar metachromatism. The body of the micro-organism stained faintly and seemed to have a slightly beaded appearance. Occasionally long curved threadlike forms were seen. It possessed no capsule or flagellum, was nonmotile and did not form spores. It grew best in freshly boiled, rapidly cooled mediums containing sterile rabbit blood. The colonies on anaerobic blood agar plates were mucoid, grayish and opalescent, and produced hemolysis. Moreover, when the micro-organism had been planted on blood agar plates and incubated aerobically, hemolysis could be detected even in the absence of visible growth. Cultures in fluid and semisolid mediums with and without blood gave off a putrid odor. Growth occurred in gelatin with a flocculent sediment, but the gelatin was not liquefied. Indol was produced in the usual test mediums. Hydrogen sulfide was not formed. Milk was peptonized but not coagulated. Litmus milk was partly decolorized. Gas and acid were produced by this micro-organism in a semisolid medium containing dextrose and maltose. Lactose, sucrose and mannite were apparently unaffected.

The micro-organism grew best at 37 C. but grew also at room temperatures. Its viability was maintained in the presence of bile, but its virulence for rabbits was reduced greatly (see subsequent section). Its virulence was also reduced by exposures to air for relatively short periods (see subsequent section).

*Pathogenicity.*—A subcutaneous injection of a virulent culture into the groin of a rabbit was followed in two days by a localized firm swelling which contained a large, sharply circumscribed area of soft white cheesy necrosis. Histologically, the area of necrosis had a granular amorphous appearance with much basophilic nuclear debris. It was sharply demarcated by an outer zone of acidophilic necrosis. The surrounding connective tissue and muscle were edematous and infiltrated with moderate numbers of polymorphonuclear and mononuclear leukocytes. Small areas of necrosis were occasionally found in the liver after subcutaneous inoculation of a virulent culture. Injections into mice were followed by similar sharply circumscribed necrotic lesions. Intratracheal injections into the lungs of rabbits were almost uniformly followed by necrotic lesions of a specific type. These are described in detail in a subsequent section.

*Classification of the Micro-Organism.*—From the foregoing description it is difficult to establish the exact taxonomic relationships of the bacillus. In many respects it resembles closely *B. necrophorus* (Schmorl), according to descriptions of the latter as collated from many sources by Weinberg.<sup>6</sup> The essential features of *B. necrophorus* are its polymorphism and metachromatic staining, its vitality under unfavorable conditions of cultivation in artificial mediums, its

5. Cohen (footnotes 1 and 4).

6. Weinberg, M.; Nativelle, R., and Prévot, A. R.: *Les microbes anaérobies*, Paris, Masson & Cie, 1937.

decolorization of litmus milk with slow curdling, its failure to attack gelatin, its production of indol and failure to produce hydrogen sulfide, and its fermentation of sugars. The pathogenicity of *B. necrophorus* for rabbits and mice is marked, according to Weinberg, the characteristic process being putrefactive necrosis at the site of local infection, followed by necrotic lesions of the viscera, especially the liver and the lungs, the bacillus being transported via the blood stream. As stated by him, the "abscesses" are rarely simply suppurative but are usually gangrenous in type, giving rise to the typical classic appearance of "necrobacillosis of the liver or lung." Other animals are less susceptible. It has been isolated from various gangrenous lesions in man,<sup>7</sup> one of the first recorded cases of its isolation being that from necrotic pharyngitis and pneumonia in an infant (Ellerman<sup>8</sup>). It has been found in gangrenous stomatitis, subcutaneous phlegmonous inflammation, puerperal infection, ulcerative colitis and erosive balanitis.<sup>9</sup> Shaw<sup>9</sup> found it in the pus of a pulmonary abscess.

Beveridge<sup>10</sup> studied twelve strains of *B. necrophorus* and found two serologic groups. Minor variations among the strains were found in the fermentation of sugars, the coagulation of milk or serum and the viability on exposure to air. As also noted by others, the histologic features of the lesions were characteristic for the species. Although the organism was highly pathogenic for rabbits, its pathogenicity for other animals was variable, and it often figured merely as a saprophyte. Its pathogenicity in guinea pigs and human beings was greatly enhanced when it occurred in association with other micro-organisms, such as cocci (Schmorl<sup>11</sup>). According to Beveridge, its ability to produce lesions appeared to be largely due to the necrosing properties of its endotoxin.

#### EXPERIMENTAL METHODS

*Method of Intratracheal Inoculation.*—The rabbits employed were of healthy stock, weighing 1.5 to 2.5 Kg. The ventral region of the neck was shaved, and a depilatory was applied to remove the last vestige of hair. The animal was tied to a board with its ventral surface up and its head held back to expose the shaved surface. No anesthetic was employed. Iodine was used for disinfection of the skin, and sterile technic was observed throughout. A linear incision was made over the trachea. The needle of a syringe containing the inoculum was gently insinuated between two rings and the desired amount of the inoculum was forced into the trachea; the needle was then quickly withdrawn, a metal clip or a single silk suture applied to the edges of the skin for approximate closure and the rabbit quickly lifted by the ears to prevent passage of the fluid into the pharynx and nose. The animal was then gently swung from side to side, and its thorax was patted with the hand, to insure a flow of the injected liquid into the lower areas of the lung.

*Methods of Examination of Postmortem Material.*—At suitable intervals after inoculation, the rabbits were killed by injection of 20 cc. of air into the marginal vein of the ear, and the thorax was opened under aseptic conditions.

7. Cunningham, J. S.: Arch. Path. 9:843, 1930. Shaw, F. W.: Zentralbl. f. Bakt. (Abt. 1) 129:132, 1933.

8. Ellerman, V.: Zentralbl. f. Bakt. (Abt. 1) 38:383, 1905.

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The lungs and the mediastinal structures, including the heart, were removed in one piece and kept in a sterile Petri dish until aerobic and anaerobic cultures had been made. Following this the organs were dissected and the gross findings noted, and material was taken for histologic study, a 4 per cent solution of formaldehyde being used as fixative.

*Preparation of an Inoculum of Fixed Virulence.*—Rabbits were inoculated subcutaneously in the groin with 1 to 2 cc. of a viable culture. The production of a large swelling signaled the formation of an abscess containing large numbers of the bacteria of enhanced virulence. Aspirated material was inoculated into Smith-Noguchi medium containing 1 cc. of sterile defibrinated rabbit blood. The former was prepared by adding to ascitic fluid 1 per cent dextrose and 1 per cent broth; 10 cc. of the mixture was poured into a long narrow test tube, 20 by 1.5 cm., and a piece of fresh sterile rabbit kidney was added. The tube was incubated overnight to make certain of its sterility. After inoculation of the medium with the anaerobe, sterile petrolatum was added and the tube incubated for forty-eight hours. At the same time blood agar plates of  $pH$  7.4 were also inoculated with a drop of the same culture and incubated aerobically and anaerobically for the same period of forty-eight hours, to rule out contamination by other micro-organisms.

#### CONTROL STUDIES EMPLOYING FULLY VIRULENT CULTURES: THE PATHOGENIC MECHANISM

In the original experiments, lesions were produced in 23 of the 27 rabbits given intratracheal injections of 2 cc. of the forty-eight hour culture just described. In later experiments a similarly high incidence of lesions was obtained with the same strain of anaerobes after it had been carried in subculture for nearly eight years. Morphologically, the lesions in the lungs caused by the original culture and those caused by the subculture were identical.

Specimens were obtained at varying intervals after intratracheal inoculation. The lesion caused by this anaerobe had a characteristic appearance, which made its recognition easy at a glance as early as twenty-four hours after inoculation. Its evolution was fairly consistent, and an approximation of its age was often possible on the basis of the morphologic features alone. For purposes of description the five day stage is selected as embodying the salient features in their most typical form.

In the unopened lung the specific lesion was detected as a sharply circumscribed, raised consolidation, lobular or sublobar in extent, bulging at one or more points on the pleural surface (fig. 1). In most instances it was solitary; in the remaining ones it appeared in closely grouped or confluent areas of consolidation involving one or more lobes, homolaterally or bilaterally. The pleural surface was covered with grayish fibrin and presented beneath this a dirty, grayish white discoloration where the underlying lesion had extended to the surface. Congestion and hemorrhage were seen within these areas and peripherally, setting off the lesion from the surrounding uninvolved regions.

On cut section (fig. 2A) the sharp demarcation of the lesion was clearly disclosed. The lesion appeared in various sizes and shapes, often with a serpiginous outline but with a general tendency to assume a pyramidal shape with the base at the pleura. Separate areas appeared to be all of the same age. Two zones could be distinguished in the lesion, an outer compact zone, more nearly solid and of a pearly white color, and an enclosed softer area of darker



Fig. 1.—Rabbit lung exhibiting a typical lesion. This was seen five days after intratracheal injection of a virulent culture. Note the uniform consolidation extending to the pleura.

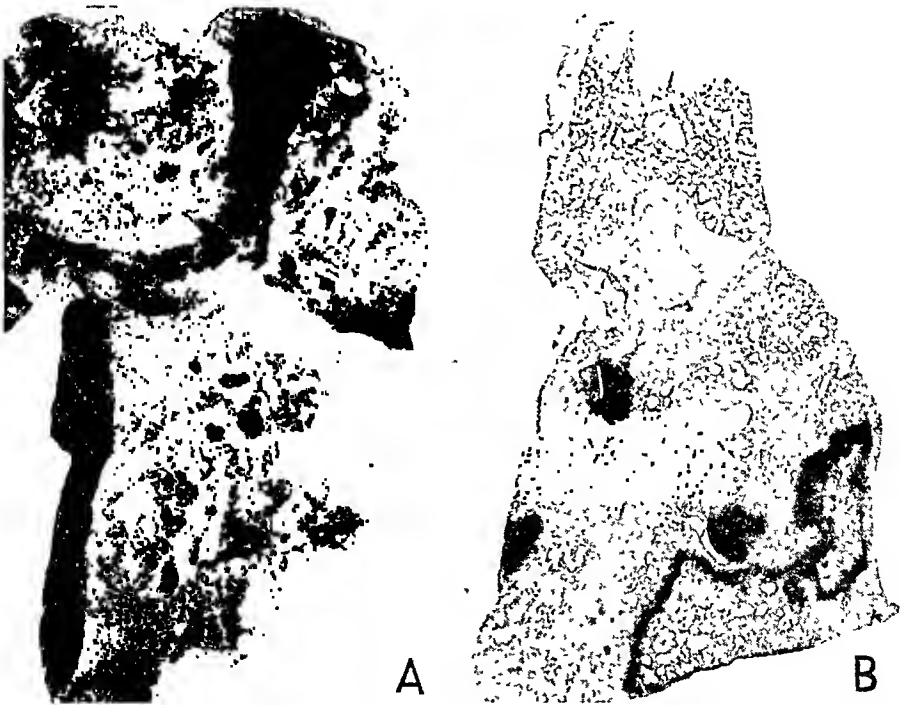


Fig. 2.—*A*, rabbit's lung opened to show the sharp demarcation of the specific lesion. Note the zonulation (marginal dense white zone with inner area of softening); *B*, low power photomicrograph of the specific lesion (hematoxylin-eosin stain).

color. The surrounding parenchyma presented a zone of congestion, edema and slight consolidation, fading gradually into normal tissue.

The histologic features corresponded well with the macroscopic observations. In each rabbit the lesion was of uniform development in all parts, apparent not only in separate areas of involvement but also in the various components of the individual lesion. In the parenchyma intervening between separate lesions or between separate portions of a large single lesion there were no evidences of continuing extension of the process. Nor were metastases found. It was clear that the final extent of the lesion had been determined in its early stages, probably as a result of rapid demarcation.

In addition to the sharp demarcation of the lesion, its other principal histologic features were its dense outer margin of leukocytic exudation and its uniform necrosis.

The marginal zone of leukocytic exudation produced the most marked tincorial change in the sections. This zone was about 0.5 to 0.7 mm. in width in typical areas and was deep purple with the hematoxylin-eosin stain because of the compact infiltration of polymorphonuclear leukocytes. The polymorphous character of the lesion was therefore best appreciated from this zone, which outlined the lesion much as colored boundaries outline geographic areas on a map (fig. 2 *B*). Under higher magnification (fig. 3 *A*) the exudate within this zone appeared as dense plugs of necrobiotic and necrotic leukocytes filling the lumens of collapsed necrotic alveoli. Fibrin and hemorrhage were scanty or lacking.

A narrow line of acidophilic necrosis formed the outermost zone of the lesion. It measured 0.3 mm. or less in width and in places was barely recognizable.

Coarser structures, e. g., bronchi, larger blood vessels and fibrous septums, which passed across the separate zones of the lesion underwent changes corresponding to each zone successively traversed (fig. 3 *B*).

The large island of inflamed parenchyma enclosed by these zones exhibited simple ischemic necrosis, in which the alveolar framework was well preserved and the capillaries and the larger blood vessels were patent. The bronchioles were patent, but the alveoli were moderately atelectatic. These structures except for a basophilic nuclear debris were faintly eosinophilic. Erythrocytes were laked. Scattered necrotic leukocytes were seen, but there was no marked exudation of leukocytes such as that seen in the marginal zones of the lesion.

None of these features was found in the remaining lung tissue. The latter appeared essentially normal except for a few areas of mild atelectasis. The parenchyma immediately adjacent to the lesion presented changes of minimal degree, consisting of mild atelectasis with proliferation and vacuolation of alveolar epithelium, slight edema and few leukocytes.

*Evolution of the Specific Lesion.*—As early as five and one-half hours after intratracheal inoculation, patchy red consolidation in the lung was detected. Histologically, there was dense leukocytic exudation within numerous areas of atelectasis. The alveolar septums showed beginning acidophilic necrosis, and necrobiotic changes were seen in some of the leukocytes and proliferated alveolar phagocytes. The most intense exudation was associated with the most extensive atelectasis. The cause of the latter was not evident inasmuch as the bronchi and larger bronchioles were widely patent and almost entirely free of exudate or other material. There were no recognizable traces of the fluid inoculum intro-

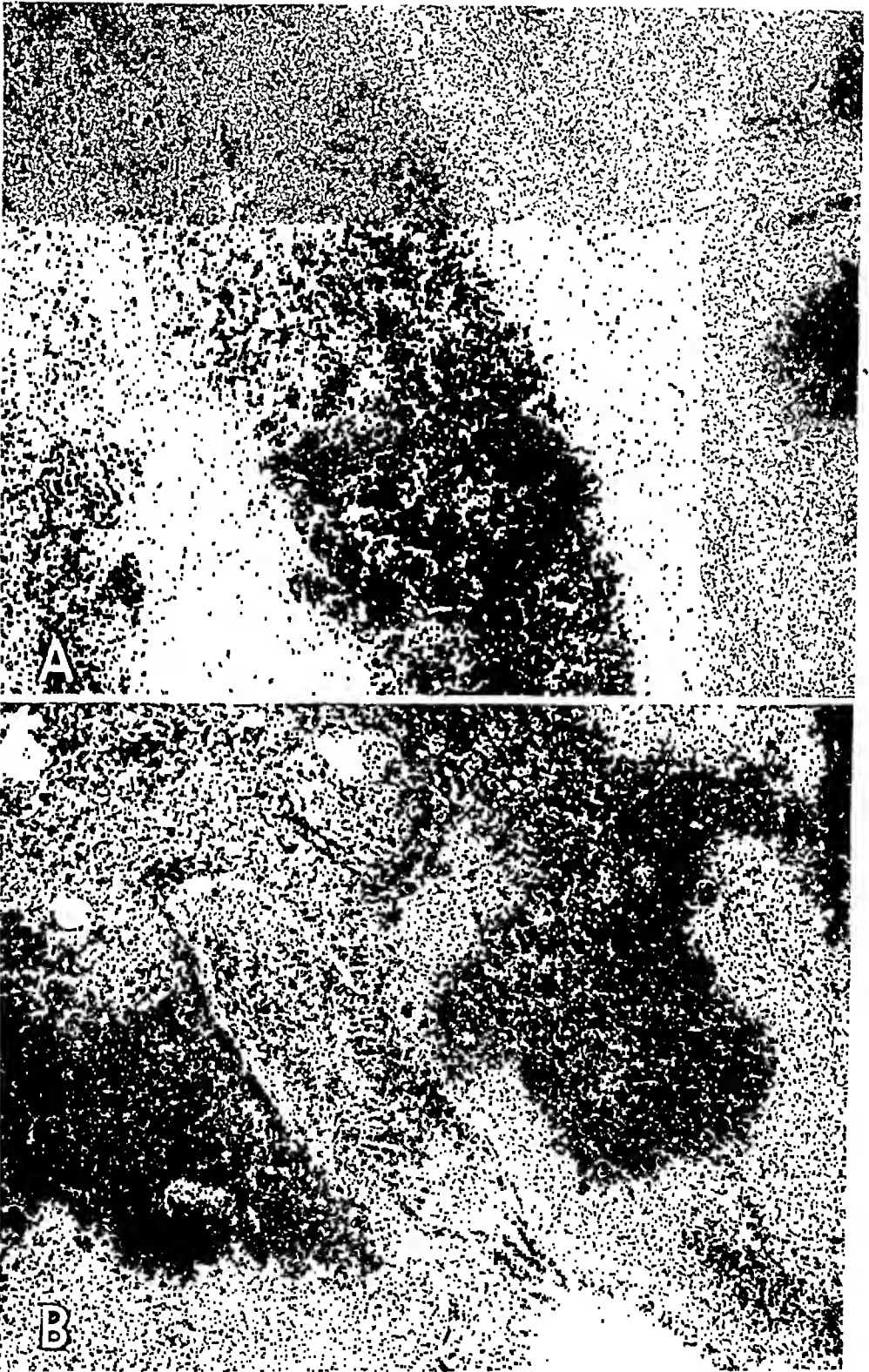


Fig. 3.—*A*, photomicrograph showing the dense leukocytic infiltration of the marginal zone of the lesion and ischemic necrosis of the enclosed parenchyma; *B*, photomicrograph showing involvement of a small bronchus at its point of emergence through the marginal zone.

duced intratracheally, and it was impossible to detect the particulate elements (bacteria) of the inoculum in the areas of consolidation.

Twenty-four hours after inoculation the lungs had well marked areas of consolidation bulging on their pleural surfaces at one or more points. In shape, distribution and size, these conformed to the description of the typical lesion given previously. The color was dusky purplish red, with relatively little of the grayish tinge seen in later lesions. They were less firm in texture than the latter and were less elevated above the pleural surface or the cut surface. The pleura was mottled red and lusterless.

Histologically, the lesion, whether solitary or multiple, presented the typical sharp demarcation. Three principal elements could be defined at this stage: (a) atelectasis, (b) leukocytic exudation and (c) necrosis.

Atelectasis, both patchy and confluent, appeared to be a constant feature. Leukocytic exudation or necrosis was mild or absent where atelectasis was slight and correspondingly marked where atelectasis was conspicuous. The necrosis was of less uniform extent than the exudation of leukocytes, and both processes ended abruptly in a sharp outer line of demarcation. The type of necrosis in the enclosed areas differed from that in the marginal zone, appearing to be the direct result of the ischemia caused by the choking of the alveolar capillaries and other nutrient blood vessels within the zone of marginal necrosis.

In the three day old lesion all the elements of the specific lesion were clearly defined. These comprised the marginal leukocytic zone, the central enclosed area of ischemic necrosis and the acidophilic necrosis involving the marginal zone, as well as the narrow line of demarcation beyond.

In older lesions, nuclear chromatolysis of leukocytes was further advanced, and slight organization appeared in the surrounding parenchyma. In some animals a considerable pleural empyema was found on the seventh day.

In 2 animals that lived about four weeks, the entire lesion underwent liquefaction and took the form of an encapsulated abscess from which the bacillus was recovered in pure culture.

*Analysis of the Pathogenic Mechanism.*—(a) Role of Atelectasis: The high incidence of positive results following single intratracheal inoculations of cultures of this strict anaerobe bears witness to the micro-organism's ability to create for itself an anaerobic environment within the lung. It is of interest, therefore, that the initial phases of the lesion were characterized by atelectasis, which determined its ultimate size and shape and which formed the matrix for the leukocytic exudation and the necrosis. As a rule the bronchi and larger bronchioles were found widely patent. From the standpoint of morphology alone it was not possible to draw any conclusions regarding the mechanism of the atelectasis, i. e. whether the collapse was caused by simple mechanical blockage or by reflex bronchiolar spasm in response to some irritant. The occurrence of atelectasis within the first few hours after intratracheal injection of the inoculum and the equally early occurrence of leukocytic exudation and beginning necrosis indicate that the anaerobe was endowed with pathogenic properties at the moment of transfer from the culture



tube to the lung tissue, even before its viability within the lung was demonstrable. It was deduced that its survival and activity in producing a specific pulmonary lesion were probably bound up with its ability to bring about immediate atelectasis. To test this hypothesis further, the following experiments were carried out (1941):

1. Forced inhalation of carbon dioxide. Each of 4 rabbits was given a large dose (4 cc.) of the inoculum intratracheally. At thirty minute intervals the animals were made to inhale a fairly high concentration of carbon dioxide mixed with air for about ten minutes. In each case respiration appeared quicker or deeper or both. The procedure was repeated ten or twelve times. The animals were killed and the lungs examined in the usual manner. In 3 animals no important differences were found in the type or the extent of the lesions except that grossly the lungs appeared slightly fluffy and the lesions appeared small and frequently confined to the hilus and deeper portions of the lungs. In the fourth rabbit small scattered areas of consolidation were found which on histologic study were found to be areas of dense atelectasis with only trifling leukocytic exudation or necrosis.

2. Forced inhalation of oxygen (95 per cent) and carbon dioxide (5 per cent). Four more rabbits were subjected to the same procedure except that a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was substituted for the mixture of carbon dioxide and air. In 1 rabbit the lungs appeared normal at autopsy. In the others the lungs were uniformly well inflated and of normal color except for a few small scattered areas of dark red consolidation. The latter when examined histologically varied considerably. In 1 rabbit a few of these areas presented the typical necrotic lesion, while others were composed of densely atelectatic alveoli in which the lining epithelium had begun to swell and desquamate. In another rabbit a single small necrotic lesion and several areas of consolidation of atelectatic type were seen. In the fourth rabbit the areas of consolidation were all of the atelectatic type except for a few minute foci of leukocytic exudation and beginning necrosis.

In a subsequent experiment 6 rabbits were inoculated in this manner and supplied with the same mixture of oxygen and carbon dioxide, but these animals rebreathed the mixed gases from an improvised rubber mask (a thin rubber glove was employed) under mildly positive pressure. Treatments were begun thirty to sixty minutes after inoculation and were maintained for ten minutes and repeated one to three times afterward at varying intervals. The positive autopsy findings in these rabbits were even less marked than those in the preceding series. In 3 rabbits minute patches of dark red atelectatic consolidation were the only finding; in 2 others microscopic foci of necrosis were present in addition. The sixth rabbit was found dead, and the lungs showed merely hypostatic congestion and edema. In none of these animals was the specific lesion recognizable grossly.

(b) Role of Bacterial Toxic Factors: As mentioned, Cohen<sup>4</sup> studied this and other anaerobes isolated from putrid abscesses of lungs for toxins capable of eliciting the Shwartzman phenomenon in the rabbit's skin. He further established a correlation between the capacity to

produce a potent skin-preparatory toxin and the necrotizing activity in the rabbit's lung.

The experiments reported in following paragraphs represent an attempt to prove that the capacity of this particular anaerobe to produce a lung-preparatory toxin is correlated with the natural pathogenicity of the organism for the lung. For the lung-preparatory factor live virulent and avirulent cultures of this anaerobe were employed, also heat-killed cultures and culture filtrates. For the provocative factor (nonspecific), given intravenously, toxin of proved potency from homologous or from heterologous sources was employed. The description of the methods of preparing, titrating and administering homologous toxin (i. e., toxin produced by this organism) and heterologous toxin (typhoid toxin, meningococcus toxin) is beyond the scope of this paper; the reader is referred to the original publications.<sup>12</sup> Every toxin employed was tested for potency by means of the Shwartzman phenomenon in the rabbit's skin.

In preliminary experiments (series 1) 50 rabbits were inoculated intratracheally with a virulent culture of the necrophorus-like anaerobe, and 22 of these were set aside as controls. The remaining 28 rabbits received varying doses of toxin intravenously after twenty-four hours, followed in some cases by additional doses at twenty-four hour intervals. The inoculums were either twenty-four or forty-eight hour cultures and were given in doses of either 1 or 2 cc. intratracheally. The toxin was either homologous, given intravenously in doses of 2 to 10 cc. per kilogram of body weight, or heterologous (*B. typhosus* toxin), given in doses of 5 cc. per kilogram of body weight.

The results were as follows: Of the 28 rabbits receiving toxin intravenously, 24 presented lesions of the lungs at autopsy. No significant differences were noted whether 1 cc. or 2 cc. of inoculum had been given intratracheally, or whether homologous or heterologous toxin had been employed. The number of rabbits used was too small, however, to permit conclusions. Of the 22 rabbits that failed to receive toxin intravenously, 14 presented lesions at autopsy.

On the basis of incidence alone, the slightly higher number of positive results in animals receiving toxin did not appear to be decisive. A difference could be appreciated, however, in the appearance of the lesions. On gross inspection the lesions of the series receiving toxin appeared larger. Microscopically, they seemed to exhibit a greater intensity of leukocytic infiltration and of necrosis. In other respects the lesions in both series were identical.

The incidence of lesions in the control series (14 of 22 rabbits) was somewhat smaller than that in an earlier series (23 of 27 rabbits). This falling off of virulence suggested an attenuating effect of repeated subcultures in artificial mediums. The apparent restoration to maximum virulence on the intravenous injection of the toxin in the toxin series (24 of 28 rabbits) prompted Cohen to undertake a deliberate attenuation of this anaerobe in order to sharpen the contrast between the results with and without the intravenous injection of toxin.

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12. Shwartzman.<sup>3a</sup> Cohen.<sup>4</sup>

The results of these and subsequent experiments are tabulated here:

I. Intratracheal injection of attenuated micro-organisms combined with intravenous injection of homologous or heterologous toxin (experiments of Dr. Cohen)

A. Slight attenuation (simple subculturing)

	Rabbits Showing Pulmonary Lesions	Total Number of Rabbits
Controls (without toxin).....	14	22
Rabbits that received toxin intravenously..	24	28

(Lesions were more extensive grossly; histologically, they showed more leukocytes and necrosis, and the surrounding parenchyma, more fibrin and edema.)

B. Moderate attenuation (repeated subculturing in artificial mediums, exposure to room temperature, cultivation in absence of blood, etc.)

Controls (without toxin).....	5	14
Rabbits that received toxin intravenously..	9	15

(Lesions were of increased size, number and intensity.)

C. Complete attenuation (exposure to room temperature for one month, subculturing in presence of homologous antiserum produced in dog, also subculturing in presence of bile.)

Controls (without toxin).....	0	45
Rabbits that received toxin intravenously..	11	28

II. Intratracheal injection of heat-killed cultures

Controls (without toxin).....	0	7
Rabbits that received toxin intravenously..	0	6

III. Intratracheal injection of culture filtrate

Rabbits that received toxin intravenously..	0	4
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From these results it appears that the intravenous injection of toxin (homologous or heterologous) enhances the virulence of this anaerobe regardless of the degree of the latter's attenuation. With slightly attenuated cultures (group A) the virulence was fully restored. With completely attenuated cultures (group C) the restoration of virulence was decisive and marked, approaching that of moderately attenuated cultures (group B). Of significance, too, was the fact that the basic appearance of the lesions produced in these experiments, even with completely attenuated cultures, was the same as that of lesions caused by virulent cultures.

The results were rather striking, particularly those in which typical specific lesions were obtained with completely attenuated cultures and with heterologous toxin, and led to additional experiments. These were carried out (1941) in more or less the same form as those in group C, which was the crucial experiment.

This phase of the work was begun after an interval of nearly eight years, during which the anaerobe was carried in an unbroken series of subcultures in artificial mediums.

The biliary attenuation procedure employed in group C was selected because of its simplicity. The culture (previously tested for freedom from contamination) was transplanted into fresh Smith-Noguchi medium containing added sterile defibrinated rabbit blood and incubated for forty-eight hours. When vigorous growth and evolution of gas with a foul odor were not obtained in the first transplant, one or two additional serial subcultures were made in the same medium and with the same period of incubation. This insured cultures of high virulence as tested by intratracheal inoculation in rabbits.

The first step in attenuation consisted in transplantation into liver hormone medium.<sup>12a</sup> This culture was incubated for twenty-four hours, after which 1 cc. of sterile fresh ox bile was added to the medium and the tube set at room temperature for five days. Transplantation was then made into plain broth and the culture incubated for eight days. Either 2 cc. or 4 cc. of the inoculum was employed for intratracheal injection, and the further procedure followed the directions outlined previously.

For the intravenous injection a heterologous toxin was employed (*B. typhosus* filtrate) diluted 1:5, 1:10 or more, depending on the titer determined by previous testing in the rabbit's skin. The amount given was 1 cc. per kilogram in each rabbit, and in these experiments a single intravenous dose was given exactly twenty-four hours after the intratracheal inoculation of the culture.

The results were as follows: Thirteen of 23 rabbits receiving both injections showed lesions specific for the anaerobe. Seven other rabbits presented nonspecific patchy pneumonia with focal necrosis; 3 rabbits had no lesions. Of 14 controls receiving only the intratracheal injection, 11 had no lesions, and 3 presented nonspecific patchy pneumonia with focal necrosis. This condition was apparently caused by incidental spontaneous infection with *Bacillus lepi-septicus* and similar flora.

It appeared, therefore, that the intravenously injected toxin not only restored some of the lost virulence of the anaerobic cultures but also enhanced the virulence of spontaneous infections.

The evolution of the toxin-produced lesion was also studied, beginning three hours after the toxin was introduced intravenously into animals that had received the attenuated cultures intratracheally twenty-four hours previously. As with virulent cultures, the early findings were scattered areas of dense atelectasis

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12a. Liver hormone medium is prepared from the following materials:

Fresh liver (veal or beef, free of fat).....	1,307 Gm. (3 lb.)
Plain broth .....	4,500 cc.
Dextrose .....	45 Gm.
Tenth-normal sodium hydroxide (added after boiling) .....	45 cc.

Directions: To 3 pounds (1,307 Gm.) of fresh liver add 35 Gm. of dextrose and 3,500 cc. of plain broth. Allow the mixture to reach the boiling point. Boil slowly for fifteen minutes, stirring occasionally. Remove the liver and set aside for later use. Add the remaining 1,000 cc. of broth and 10 Gm. of dextrose. Filter through cotton into 2 Florence flasks until the broth is clear. Add 45 cc. of tenth-normal sodium hydroxide. Adjust *pH* to 7.4. Slice the liver into cubes and insert into anaerobic tubes; pour broth over the liver; pour sterile petrolatum over the surface of the broth. Autoclave at 10 pounds (4.5 Kg.) for ten minutes.

together with some leukocytic exudation and beginning necrosis. Controls, not receiving toxin intravenously, also showed patchy atelectasis with leukocytic exudation but no necrosis. Necrobiotic changes were present in the alveolar phagocytes and leukocytes in animals which had received toxin intravenously. Necrosis of the alveoli was prominent, taking the form of acidophilic swelling and fragmentation of the ground substance. The cement substance of the capillaries appeared swollen and often fused in a smudge with the rest of the alveolar septum and with the swollen and partly desquamated alveolar epithelium. Some of the capillaries were abnormally dilated and in places were broken down, with resulting hemorrhage. For the most part, they appeared contracted and more or less bloodless.

Twenty-four hours after the injection of toxin these changes were somewhat more advanced. In forty-eight hours the ground substance of the alveolar septums presented marked acidophilic swelling and foamy, granular alteration, also the capillary endothelium, the alveolar epithelium and the leukocytic exudate in the lumen. Nuclear breakdown had begun, with diffusion of chromatin into the cytoplasm. The involved area was nearly bloodless because of the choking of the capillaries by the swelling of the ground substance and of the endothelium. Vague zonulation was evident.

Four days after the injection of toxin the typical appearance of the specific lesion was apparent, including the well defined zonulation and sharp demarcation observed in the lesion produced by virulent cultures.

Controls, which received only the intratracheal injection, showed no necrosis even after several days, despite increased infiltration of leukocytes in some animals.

The effect of concentration of attenuated bacteria on lung-preparatory potency was studied: Several tubes of attenuated culture were centrifuged and the supernatant fluid discarded. The pooled sediments in doses of 4 cc. were injected intratracheally into 23 rabbits. Fifteen of these twenty-four hours after inoculation received toxin intravenously. Seven of the latter died within a few hours after receiving the toxin; the remaining 8 were killed one to five days later. Two of the 8 controls were killed three hours after intratracheal inoculation in an attempt to recover the micro-organism by culture of the lungs. These rabbits were not examined. The remaining 6 were killed and autopsies made one to two days after inoculation.

The results may be summarized as follows: Pulmonary changes were found in all rabbits on which autopsies were made regardless of whether or not toxin had been given intravenously. The findings consisted in circumscribed patches of dense atelectatic consolidation, showing histologically slight, moderate or marked exudation of leukocytes and corresponding degrees of focal acidophilic necrosis. In 1 of the 15 rabbits that had received toxin intravenously a small area characteristic of the typical lesion was seen as well. Otherwise there was no significant difference between those receiving toxin and the controls.

From these findings the conclusion was drawn that the concentration procedure robbed the attenuated anaerobe almost entirely of its capacity to prepare lung tissue for the Shwartzman phenomenon, apparently as a result of overexposure to air. The dense atelectasis with leukocytic exudation and focal necrosis within early lesions was explained as evidence of massive lysis of bacteria with liberation of toxins. This assumption was strengthened by the greatly diminished incidence of pulmonary lesions in the animals killed last. The postmortem cultures

of the lungs of all the rabbits which had received these concentrated attenuated cultures proved negative.

It was concluded, therefore, that if attenuation is carried beyond a certain point, lung-preparatory potency may be entirely lost and, conversely, that only the bacterial cells which maintain their viability in the lung are capable of preparing it for the phenomenon. The importance of strict anaerobiosis is also made clear inasmuch as a great loss of viability is shown to take place after a short exposure to air.

#### COMMENT

The present study was undertaken as part of a larger plan to investigate the genesis of pulmonary abscess, employing in part strains of organisms isolated from typical cases of the disease in human beings. A more thorough identification of these strains and a further study of their relative frequency will become necessary in subsequent investigations having as their ultimate purpose the experimental production in laboratory animals of lesions resembling the various forms of the disease in man.

The phase of the work reported here concerns the cultural characteristics and pathogenicity for the lung of a gram-negative strictly anaerobic bacillus obtained from the pus of a putrid abscess in a human lung. Within the limitations of the present study, in which no special stress was laid on bacteriologic identification, the characteristics observed seemed to relate the organism to *B. necrophorus*. It produced a foul odor in various cultural mediums and was highly virulent for rabbits when injected in pure culture subcutaneously or intratracheally. It was also virulent for mice.

From the point of view of morphology the pulmonary lesion produced by the intratracheal injection of the bacillus was remarkable for its sharp demarcation, even in its earliest stages, and for the dense atelectasis, which formed the matrix for the other features of the lesion, viz., leukocytic exudation and necrosis. The infiltration of leukocytes was peculiarly dense at the margin of the lesion, where they, with the parenchyma, quickly underwent necrobiosis. The capillary blood channels in the marginal zone became obliterated early, and as a result the enclosed parenchyma (and pleura) presented simple ischemic necrosis. Eventually the necrotic areas became sequestered and liquefied so that the entire lesion became an abscess and often involved the pleural cavity to form an empyema.

Studies of the evolution of the specific lesion in successive stages yielded evidence that this strain of anaerobic bacilli survived only in those areas of the lung where intratracheal injection brought about the degree of atelectasis and circulatory blockage needed to produce an anaerobic environment. Consequently the lesion remained confined to the atelec-

tatic area of initial implantation and hence sharply demarcated. When the development of anaerobiosis was impeded as, for example, by repeated forced inhalation of a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, the pathogenicity of the culture was diminished. Specific lesions were few and small and rarely reached the pleural surface. Often the only finding was patchy dense atelectasis with little or no acute inflammation or necrosis. The therapeutic application of this observation to the prophylaxis of anaerobic abscess of the lung in man is worthy of consideration, but the experiments described here were too few to have more than suggestive value.

The mechanism of necrotization in areas infected with this anaerobe remains the key question in a study of the pathogenicity of the organism. Cohen's views, embodied in part in this paper, stressed the Shwartzman phenomenon as the underlying principle in the necrosis of the lung, whether produced by the usual synergistic mixture or by a single species of organisms endowed with primary virulence. Although the classic Shwartzman phenomenon is described as extremely severe hemorrhagic necrosis, hemorrhage is not inevitably present, as shown in experiments in certain organs and tissues.<sup>3b</sup>

Cohen,<sup>4</sup> in his first approach to the analysis of putrid abscess of the lung through the Shwartzman phenomenon, demonstrated that pure cultures of the usual types of anaerobes recovered from human lungs thus involved not only failed on intratracheal injection to produce abscess of the lung in rabbits but were unable to produce *in vitro* toxins that were potent as skin-preparatory factors for the phenomenon. On the other hand, certain symbiotic mixtures of these anaerobes, grown together *in vitro*, yielded toxins possessing a considerable degree of skin-preparatory potency. Toxins with provocative potency were regularly found in the pure cultures.

Later work revealed in certain cases of pulmonary abscess a type of anaerobic bacilli which produced toxin with preparatory potency as well as provocative potency when grown in pure culture and which was capable, furthermore, of producing severe necrotizing lesions in the lungs of rabbits when injected intratracheally. The correlation thus found between preparatory potency and pathogenicity led to the assumption that the latter was dependent on the *in vivo* production by this particular type of toxic factors capable both of preparing tissue for the Shwartzman phenomenon and of provoking this phenomenon in the course of infection. These factors, in turn, were assumed to react synergistically, the preparatory factor sensitizing the tissue perivascularly, the provocative factor entering the blood stream to revert to the prepared site by the endovascular route, in accordance with the requirements for elicitation of the phenomenon.<sup>3b</sup>

Experimental proof of these assumptions was facilitated by the fact that in the production of the phenomenon the provocative factors bear

no intrinsic relation in kind or degree to the preparatory factors.<sup>13</sup> A micro-organism may produce considerable preparatory factor, yet relatively little provocative factor, and vice versa.

In the experiments now recorded it was demonstrated that the intravenous injection of either homologous or heterologous toxin twenty-four hours after intratracheal inoculation of attenuated cultures of this anaerobe results in necrotic lesions indistinguishable from the specific lesion produced by virulent cultures. Controls presented merely foci of dense atelectasis and mild leukocytic exudation.<sup>14</sup> The conclusion seems warranted that as a result of attenuation provocative potency is lost but preparatory potency is largely retained.

The question might be asked whether the focal atelectasis and the mild exudation of leukocytes seen in the lungs of controls are the visible representation of the preparation of the lung for the Shwartzman phenomenon. If so, might they not be nonspecific and reproducible by any micro-organism capable of evoking inflammation in the lung?

These questions can be answered by reference to the experiments with cultures which were killed by heat or weakened by excessive exposure to air, in which it was found that lung-preparatory potency was completely lost. In one experiment heavy intratracheal inoculation of air-weakened, attenuated cultures produced fairly extensive atelectasis and acute inflammation with multiple foci of necrosis, but the rabbits receiving toxin intravenously did not show significant effects of the added toxin as compared with controls. These lesions were attributed to irritant toxins liberated by bacteria undergoing lysis en masse. The relative failure of preparation of the lung on intratracheal injection of nonviable cultures or of culture filtrates must be laid to failure of interstitial (perivascular) union between toxin and tissue. On the other hand, the living organism, endowed with natural invasiveness even though attenuated, presumably achieves this in the course of implantation. Moreover, it appears that the degree of inflammation, atelectasis or other change produced by the anaerobe does not necessarily correspond with its tissue-preparatory potency. In all probability the latter alone is type specific, and after the implantation of the attenuated form the intravenous injection of any potent provocative factor results in a necrotic lesion typical of the virulent form.

An explanation of necrosis brought about by bacterial synergism, as in the ordinary case of putrid abscess of the lung, in which one finds a

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13. Shwartzman,<sup>3</sup> chap. 2, p. 31.

14. A corresponding lack of local reaction was observed in each of 2 rabbits twenty-four hours after 0.25 cc. of a similarly prepared culture of the anaerobe had been injected intradermally. Four hours after these rabbits had been given typhoid toxin intravenously, the previously injected skin sites presented lesions typical of the Shwartzman phenomenon and having an intensity recorded as + + + +.



mixture of several types of anaerobes that individually are nonpathogenic, is also supplied by these experimental results. The usual anaerobic bacilli of this group, although individually lacking in preparatory factors, are a rich source of provocative factor, as previously demonstrated.<sup>5</sup> The anaerobic streptococcus and the anaerobic diphtheroid which are found regularly in cases of putrid abscess of the lung may be suspected as the source of preparatory factor despite their apparent innocuousness. Proof of this is still lacking, and further work is contemplated along this line.

#### SUMMARY

Previous studies have demonstrated that pure cultures of the anaerobic micro-organisms recovered from the usual mixed flora of putrid abscess of the lung in man are incapable of reproducing the lesion in animal experiments. Exceptionally, certain anaerobic bacilli have been found which are pathogenic in pure culture. One such bacillus is described in the present report with special reference to the mechanism of its action when it is introduced intratracheally into the lung of a rabbit.

Culturally and otherwise it resembled *B. necrophorus*. From the point of view of morphology the lesion caused by it appeared to be specific. In the genesis of this lesion, studied in various stages, the primary incident was seen to be lobular atelectasis, which provided an anaerobic environment for the survival of the bacterium. Forced inhalation of a mixture of oxygen and carbon dioxide largely prevented the development of the lesion. Dense leukocytic infiltration and necrosis quickly followed the primary atelectasis and formed a sharply demarcated consolidation ending in abscess with or without empyema.

The factors in the production of necrosis by this anaerobe were studied by application of the principles of the Shwartzman phenomenon. Earlier experiments had shown that pure cultures of the usual anaerobic bacilli found in putrid abscess of the lung failed to produce potent skin-preparatory toxins for the phenomenon, although active in producing potent provocative toxins. Conversely, the exceptional anaerobic bacilli which were pathogenic in pure culture were capable likewise of producing potent skin-preparatory toxins.

In the experiments reported here, the capacity to produce a necrotizing lesion in the lung of the rabbit was abolished by subculturing the anaerobe in the presence of bile. Nevertheless the capacity to produce potent skin-preparatory and lung-preparatory toxins endured, as proved by the production of necrotic lesions in prepared sites in the skin and the lungs, respectively, by injecting potent provocative toxins intravenously at a suitable interval, in accordance with the requirements for elicitation of the Shwartzman phenomenon. The lesions thereby produced in the lungs were identical with those produced in a primary manner by virulent cultures.

# IMMATURE BOTRYOID TUMORS OF THE CERVIX

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In 1867 Weber<sup>1</sup> described the first of a series of uncommon and peculiar tumors of the cervix which subsequently received various names, such as "sarcoma botryoides," "myxochondrosarcoma," "myosarcoma striocellulare uteri," "mixed tumor" and "dyontogenetic tumor" (one type). Most of these tumors have been called sarcoma. In the present state of knowledge it might still be best to refer to them according to their gross appearance, as in the instance of sarcoma botryoides.<sup>2</sup> Yet this is not entirely satisfactory either, since there still remains the possibility that these growths are teratomatous, a likelihood that has not been generally entertained. Some of these immature, rapidly growing polypoid tumors, although grossly appearing alike, may be quite different histologically, some seeming to be pure sarcoma, containing round or spindle cells or both, while others in addition contain embryonal striated muscle and cartilage. These are the so-called mixed tumors or mixed mesodermal tumors. Furthermore, some tumors have been described containing these foreign histologic elements that grossly were not polypoid at all. McFarland<sup>3</sup> in an interesting paper discussed the dyontogenetic tumors. Only those growths showing a botryoid appearance are discussed in the present paper.

## LOCATION AND AGE INCIDENCE

There are three possible places of origin: the corpus uteri, the cervix and the vagina. There is a definite difference in the average age at which the tumor is first seen, and in the number of cases reported from each of the three sites, although accurate statistics are not possible. The largest single group apparently arises from the vagina,<sup>4</sup> while there is a difference of opinion as to the numbers arising from the cervix and the corpus.<sup>5</sup>

1. Weber, O.: Virchows Arch. f. path. Anat. **39**:216, 1867.

2. Pfannenstiel, J.: Virchows Arch. f. path. Anat. **127**:305, 1892.

3. McFarland, J.: Surg., Gynec. & Obst. **61**:42, 1935.

4. McFarland, J.: Am. J. M. Sc. **141**:570, 1911.

5. Meikle, G. J.: J. Obst. & Gynaec. Brit. Emp. **43**:821, 1936. Shaw, W.: *ibid.* **35**:498, 1928.

TABLE 1.—*Immature Botryoid Tumors of the Cervix Containing Heterologous Tissue*

Author	Age	Description of Tumor	Metastases	Outcome
Weber <sup>1</sup> .....	45	From anterior lip of cervix. Spindle cells and embryonic striated muscle cells	Parametrium; uterine cavity	Death in 1 year; perforation of mass into peritoneum
Kunert <sup>13</sup> .....	35	From cervix. Large round cells and single strands of striated muscle fibers	Parametria; vault of vagina; 7th and 8th left ribs	Death in 1½ years; cachexia
Thiele, M.: Ztschr. f. Geburtsh. u. Gynäk. 1 : 460, 1877	45	From cervix. Myxomatous and fibrous tissue; cartilage	.....	Death in 3 years; exhaustion
Rein <sup>10b</sup> .....	21	From anterior and posterior lips of cervix. Round, spindle and star-shaped cells; tissue myxomatous; cartilage	Left parametrium; vault of vagina; left pelvic lymph nodes	Death in 2 years; peritonitis; perforation of metastases into left parametrium
Müller, W.: Arch. f. Gynäk. 30 : 249, 1887	24	From cervix. Round and spindle cells; striped muscle	Left broad ligament; myxomatous tissue; cartilage	Death 1 year after operation; peritonitis
Pernlee <sup>8a</sup> .....	17	From cervix. Myxomatous tissue; round and spindle cells; striated muscle; cartilage in recurrent tumor	Endometrium; free peritoneal part of pelvis, between symphysis and bladder	Death in 1½ years; cachexia, hypostatic pneumonia
Richter, cited by Peham, H.: Monatsehr. f. Geburtsh. u. Gynäk. 18 : 191, 1903	2½	From cervix. Round cells; myxomatous tissue; striated spindle cells	.....	Death after 2 years
Pfannenstiel <sup>2</sup> .....	53	From anterior wall of cervical canal. Round and spindle cells; hyaline cartilage	.....	Cachexia, recurrence of symptoms at last report
Piek, L.: Arch. f. Gynäk. 46 : 191, 1894	2½	From posterior wall of cervix. Large round cells; striated muscle	Extension to uterus	Death in 6 months; cachexia
Wilms <sup>9</sup> .....	41	From cervix. Round and spindle cells; cartilage; embryonic myxomatous tissue	Mesentery and parametrium	Death in 1½ years; perforation of uterus, peritonitis
Peham, H.: Monatsehr. f. Geburtsh. u. Gynäk. 18 : 191, 1903	18	From cervix. Striated muscle; cartilage; myxomatous tissue	Pelvic peritoneum	Death 11 months after last operation; cachexia
Kehrer, E.: Monatsehr. f. Geburtsh. u. Gynäk. 23 : 646, 1906	38	From right wall of cervix. Round and spindle cells; cartilage; bone; myxomatous tissue	.....	Death from intestinal obstruction
Michel, G., and Hoehe, L.: Compt. rend. Soc. d'obst. de Paris 9 : 44, 1907	35	From cervix. Sarcoma with cartilage	.....	Unknown
Bäcker and Minnich <sup>10</sup> .....	25	From cervix. Spindle cells; cartilage	.....	Death in 7 years; cachexia; edema of lung
McCann, F. J.: J. Obst. & Gynaec. Brit. Emp. 14 : 202, 1908	52	From cervical canal. Immature cells; cartilage	.....	Unknown
Puech, R., and Mas-sabaum, G.: J. méd. franç. 2 : 355, 1908	59	From cervix. Myxomatous tissue; cartilage; spindle cells; glandular tissue	.....	Well for 6 years. No further follow-up
Heddäus <sup>14</sup> .....	48	From cervix. Cartilage	Pleura	Death. See text
Cox, D. M., and Benischek, W. L.: Am. J. Obst. & Gynec. 16 : 28, 1928	29	From anterior lip of cervix. Bicornuate uterus, one side pregnant. Round and spindle cells; striated muscle, cartilage	.....	Death 1 year after onset
Beckmann, W.: Ztschr. f. Geburtsh. u. Gynäk. 75 : 566, 1914	22	From cervix. Round cells; cartilage; bone	Cavity of pelvis	Death 13½ months after being seen; debility
Melke <sup>5</sup> .....	48	From anterior lip of cervix. Cartilage; spindle cells	.....	No recurrence 18 months after hysterectomy
Medina, J.: Rev. de gynec. d'obst. 31 : 3, 1937	35	From anterior lip of cervix. Striated muscle	.....	Unknown

TABLE 2.—*Immature Botryoid Tumors of Cervix Not Containing Heterologous Tissue*

Author	Age	Description of Tumor	Metastases	Outcome
Spiegelberg, O.: Arch. f. Gynäk. 14: 178, 1879.....	17	From anterior lip of cervix. Spindle and round cells; hydropic intercellular substance	Corpus uteri; posterior wall of bladder	Death 1½ years after onset; postoperative peritonitis
Spiegelberg, O.: Arch. f. Gynäk. 16: 124, 1880.....	31	From posterior lip cervix. Round cells; myxomatous-like tissue	Parametrium; between vagina and rectum; vaginal vault	Death in 1½ years; bowel obstruction and peritonitis
Winkler, F. M.: Arch. f. Gynäk. 21: 309, 1883.....	47	From cervix. Large round and spindle cells; hydropic intercellular substance	Parametria; vaginal mucosa; endometrium	Death in 9 months; cachexia
Kunitz, E.: Ueber Papillome der Portio vaginalis uteri, Berlin, G. Schade, 1885	19	From cervix. Round and spindle cells; myxomatous tissue	Cervical canal; right parametrium	Not definite
Munde, P. F.: Am. J. Obst. 22: 126, 1889.....	19	From cervix. Myxomatous tissue; glands	.....	Not given
Worrall, R.: Australasian M. Gaz. 15: 292, 1896.....	23	From cervix. Myxosarcoma. No details	.....	Not given
Emmet, B. M.: Am. J. Obst. 45: 386, 1902.....	19	From posterior lip of cervix. Spindle cells. No details	.....	Death in 1 year
Curtis, H. J.: Tr. Obst. Soc. London 45: 320, 1904.....	1	Tumor filling vagina. Round and spindle cells; edematous tissue	Left iliac vessels embedded in growth	Death 2 days after operation
Phillips, J. E.: Virginia M. Semi-Monthly 9: 508, 1904-1905	15	From cervix. Spindle cell sarcoma. No details	Whole pelvis filled to brim	Death 3 months after mass in pelvis appeared
Williamson, H.: Tr. Obst. Soc. London 47: 119, 1906....	39	From anterior lip of cervix. Round and spindle cells	Parametria	Not given
Purslow, C. E.: Proc. Roy. Soc. Med. (Sect. Obst. & Gynae.) 2: 81, 1908-1909	21	From cervix. Giant cell sarcoma	Pelvis above pubis	Death 1 year after hysterectomy
Heller, J. B.: J. Obst. & Gynae. Brit. Emp. 26: 108, 1914	34	From posterior lip of cervix. Spindle cells; myxomatous tissue	.....	Death in 2½ years; cachexia
Reusch, W.: Zentralbl. f. Gynäk. 40: 37, 1916.....	16	From posterior cervical wall. Spindle cells; myxomatous tissue	.....	Not given
Jones, S. W. M.: J. Obst. & Gynae. Brit. Emp. 35: 320, 1923	18	From cervix. Spindle and round cells; myxomatous tissue	Tumor to umbilicus	Death 1 year after onset
Cox, D. M., and Denischek, W. L.: Am. J. Obst. & Gynae. 16: 28, 1923	21½ mo.	From cervix. Spindle and round cells; myxomatous tissue	Pelvis filled; hydro-nephrosis	Death 2 years after onset
Matyas, M.: Zentralbl. f. Gynäk. 55: 2739, 1931.....	25	From cervix. Spindle cells, sparsely cellular connective tissue. Patient had exophthalmic goiter	.....	Unknown

The tumors arising from the corpus tend to appear between 45 and 65 years of age,<sup>6</sup> while those of vaginal origin are mostly seen in infants and young children. The immature polypoid tumors of the cervix I have found described in the literature have been divided into those which arise from the cervix and contain heterologous elements and those in which the external appearance is the same but in which no heterologous tissue was reported. For the 22 cases in the former group the average age of incidence was 32 years, with a range of from 2¼ to 59 years. For the 16 cases in the latter group the average age was 22 years, with a range of from 1 year to 47 years. It is quite possible that many of the tumors in the second group really belong to the first, since the heterologous elements may easily be missed. The statement<sup>2</sup> that the majority of these cervical tumors occur in the age period under 20 years or in that following the menopause, with few in the intervening period, is not correct. Of the 22 cases in the first group, 12 were those of patients over 20 or under 46 years of age.

#### DESCRIPTION OF TUMORS

Classification is not easy. Cervical tumors are either diffuse or polypoid, although Ewing<sup>7</sup> has written that the latter represent a natural tendency of growth of the former and that there is no essential difference between them. McFarland<sup>3</sup> stated that the botryoid appearance results only from the amount of moisture the tumor happens to contain. Histologically, the chief distinction has been drawn between those tumors containing heterologous elements and those which are pure sarcoma. The gross classification seems the better one, for in the present state of knowledge it is simple and, further, the peculiar manner of growth deserves some emphasis.

Macroscopically, in the usual case the vagina is partially or completely filled with polypoid growths varying in size from that of a pea to that of a plum, whose origin by a narrow pedicle can be traced to one or the other lip of the cervix or to the canal. Such polyps may even be found protruding from the vulva. They are easily broken off, and the examining hand may come away holding several of them. There is much resemblance to a hydatidiform mole. The polyps are pinkish gray and look cystic, but if cut across they are found to be solid or semisolid and of a rather gelatinous consistency. They apparently arise in the subepithelial layers of the cervix.

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6. Liebow, A. A., and Tennant, R.: *Am. J. Path.* **17**:1, 1941.

7. Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1940, pp. 287-292.

Microscopically, there are two types to describe. Those of simpler composition consist in the main of spindle and round cells, one type usually predominating, while the presence of giant cells is not uncommon.

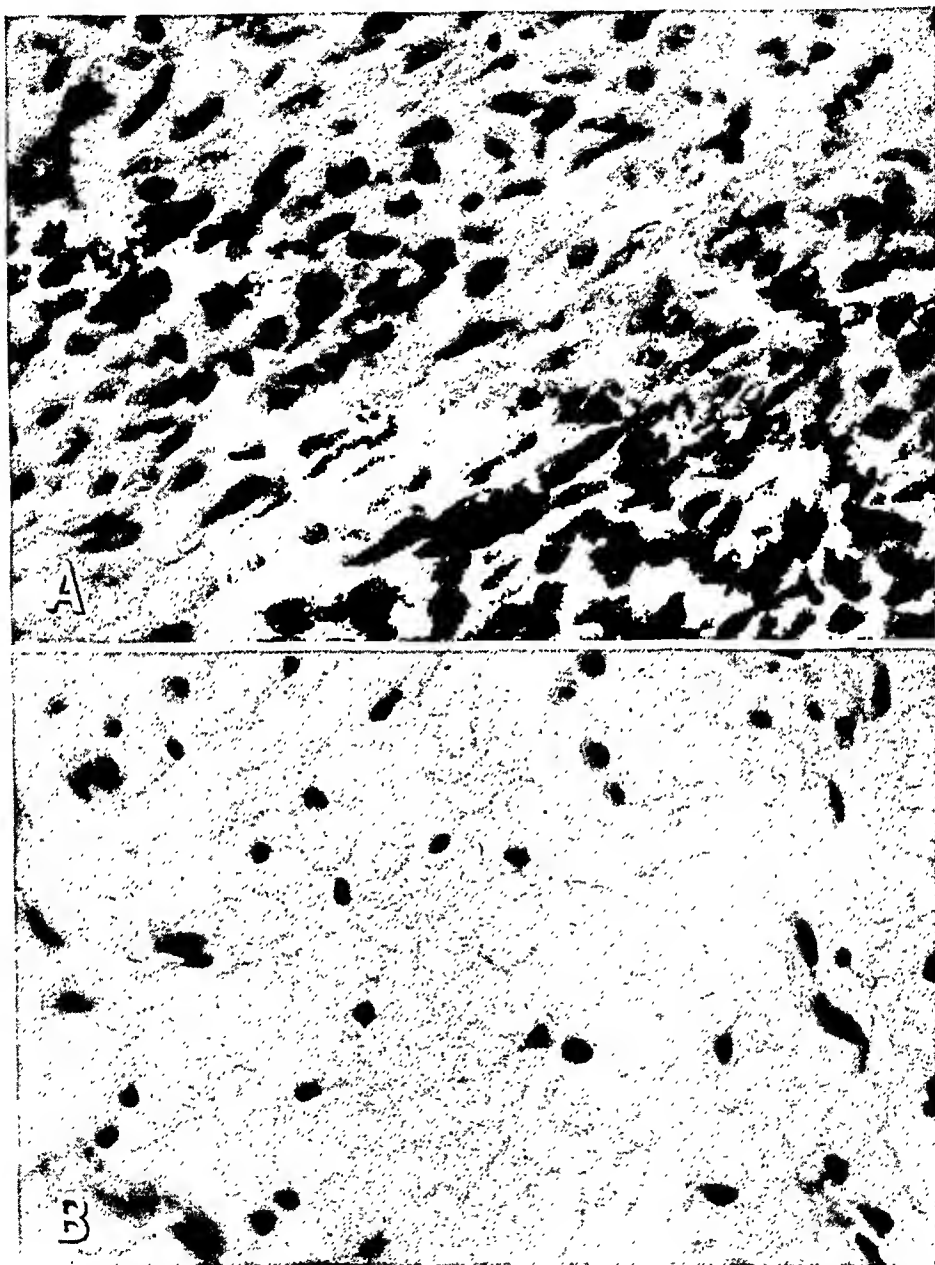


Fig. 1.—*A*, immature cells tending toward spindle formation in one portion of the tumor. Hematoxylin and eosin stain;  $\times 500$ . *B*, myxomatous-like tissue, showing paucity of cells. Hematoxylin and eosin stain;  $\times 500$ .

mon. A goodly portion of the tumor is occupied, however, by areas containing much intercellular substance and few cells. The nature of this portion of the growth has been the subject of much controversy.

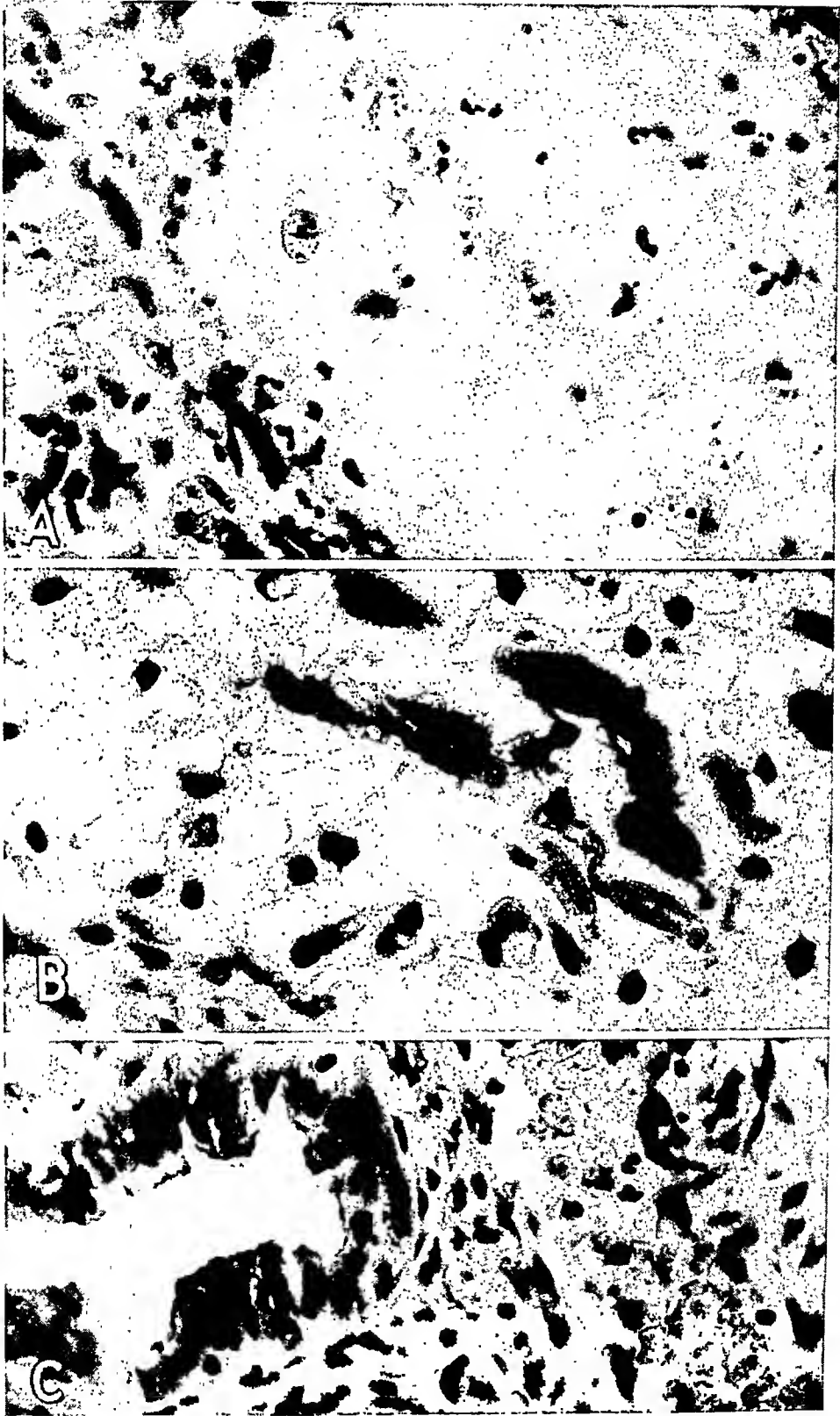


Figure 2

(See legend on opposite page)

Some consider it true myxoma.<sup>8</sup> Wilms<sup>9</sup> considered it to be embryonic mesenchymal tissue, believing this to be the fundamental constituent of the tumor, from which the other tissues are derived. Should this be true, then separation of these tumors into two groups would be quite artificial, and those listed in table 2 would represent tissue not yet showing heterologous elements but which later on might do so. Pfannenstiel<sup>2</sup> and Spiegelberg<sup>10</sup> considered that edema alone caused the appearance of this tissue. The latter based his opinion on the negative reaction for a test for mucin ("sarcoma lymphangiectaticum et hydropicum"). Whatever the exact nature of this tissue, the appearance is quite characteristic. The cell bodies are more or less star shaped or triangular with long protoplasmic threads, producing a loose network. The intercellular substance is abundant, clear or somewhat granular, sometimes eosinophilic. The cell nuclei are round or oval.

Those tumors containing heterologous elements show areas exactly as just described, with the addition here and there of patches of either islands of cartilage or strands of striated muscle cells or both, although the latter may be difficult to find. Striated muscle was noted in 10, and hyaline cartilage in 17, of the 22 cases. Both striated muscle and cartilage occurred in 5 of these cases.

Osteoid tissue is rare, having been described in only 2 cases of botryoid tumor. These tumors are vascular. One of the unusual features is the completeness of the epithelial covering, which is usually squamous epithelium. Care must be taken in examining the most superficial polyps, which may resemble a mature tumor.

The question of the presence of glandular elements is an important one. It is the consensus that these are inclusions. However, some of the acini in the sections in the case now described appear to have actively proliferating cells with heavily chromatic nuclei, but not as yet

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8. (a) Pernice, L.: Virchows Arch. f. path. Anat. **113**:46, 1888. (b) Rein, G.: Arch. f. Gynäk. **15**:187, 1880.

9. Wilms, M.: Die Mischgeschwülste der Vagina und der Cervix uteri, Leipzig, A. Georgi, 1900.

10. Spiegelberg, O.: Arch. f. Gynäk. **4**:344, 1872. •

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#### EXPLANATION OF FIGURE 2

A, very early cartilage formation. This appears more clearly under the microscope than in the photograph. Hematoxylin and eosin stain;  $\times 500$ .

B, embryonic striated muscle cells with cross striations especially evident. The tumor showed many such cells, some closely, others more loosely packed. Hematoxylin and eosin stain;  $\times 700$ .

C, small cervical gland. Note heaped-up, irregular epithelium. See text for discussion of glandular elements. Hematoxylin and eosin stain;  $\times 500$ .



truly anaplastic cells. If these glandular elements are an essential part of the tumor, it should be classified as teratoma. On the other hand, it may be that these glandular elements are stimulated to proliferation by what von Hansemann has termed "collateral hyperplasia." This change has been described as occurring in either the gland cells or the stroma of the invaded tissues and occasionally is scarcely distinguishable from neoplasia, there being perhaps some sort of trophic action on these adjacent tissues by the tumor.

Cases have been described in which there was present just one polyp, containing striated muscle or cartilage or both.<sup>11</sup> This may represent the early stage of the botryoid appearance.

#### HISTOGENESIS

The earliest considered possibility was that of metaplasia.<sup>2</sup> Some have described the transition of spindle cells to cartilage.<sup>12</sup> It is doubtful, however, if the transition between smooth and striated muscle has ever been proved.

Cohnheim's cell rest theory has been advocated, the tumor being traced to misplaced embryonal cells. Cohnheim considered that the cells originally belong to the wolffian body, but his explanation is inadequate since striated muscle is foreign to the wolffian system.

Wilms's modification of Cohnheim's theory has drawn the largest number of followers. In short, he supposed not that single cells but that undifferentiated embryonal germ tissues are split off from the region behind the renal anlage and carried toward the cloaca by the growth of the wolffian duct. He assumed, then, a common mother tissue from which cartilage as well as striated muscle may commence to grow. Of the factors that cause the growth, nothing is known. Parity plays no role in the causation of this tumor. It may well be that, as Oertel wrote in regard to another matter, "*The sarcomatous . . . manner of growth* is an expression of the conditions and environmental influences of the growth, rather than of cell derivation and character."

#### METASTASES AND EXTENSION

Distant metastasis is uncommon, while local recurrence and extension are the rule. The growth is commonly found to extend into the parametria, the broad ligaments, the pelvic peritoneum and the vagina. Death commonly results from pressure on the ureters by the tumor with hydronephrosis.

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11. Amolsch, A. L.: *Am. J. Cancer* **37**:435, 1939. Seydel, O.: *Ztschr. f. Geburtsh. u. Gynäk.* **45**:237, 1901. Spuler, R.: *Centralbl. f. allg. Path. u. path. Anat.* **16**:337, 1905.

12 Perlstein, I.: *Surg., Gynec. & Obst.* **28**:43, 1919.

Kunert<sup>13</sup> has reported metastases in the ribs. Perhaps the most interesting case is that recorded by Heddäus.<sup>14</sup> The patient was well for one and a half years after the primary tumor had been removed by vaginal hysterectomy. Then cough and right-sided thoracic pain developed, and later resection of a rib revealed 2 to 3 liters of hemorrhagic exudate and a tumor like a hydatid mole. The tumor appeared to spring from the diaphragmatic portion of the pleura. The patient died twenty-eight days later. Microscopically, the tissue resembled the primary tumor and contained many islands of cartilage. This case sheds light on the peculiar manner of growth of this tumor, in which apparently a free cavity is necessary for the development of the polypoid character, as in the vagina or the pleural cavity. Elsewhere, where no cavity exists, the tumor is solid.

#### CLINICAL FEATURES

It is possible to have extensive development of the tumor without any symptoms, even to the protrusion of the growth from the vulva. Usually, however, attention is drawn to the fact that something harmful is afoot by a watery, serosanguineous, mucoid or bloody discharge. Sooner or later this will acquire a foul odor. At times the patient will notice the passage of one or several polypoid masses, which she may insist are blood clots.

Pain is an indication that irremediable growth and damage have occurred. In the later stages vesical irritability and rectal tenesmus are common. As a later development, pressure on the ureters may cause hydronephrosis. Cachexia, anemia, a negative fluid balance and all the other evidences of declining metabolism inevitably ensue.

Physical findings will depend on the stage at which the patient presents herself, commonly enough about six months after the onset of any symptom, at which time the disease will be found to be already advanced. The appearance of the tumor has been described.

The diagnosis is made on microscopic study. Care is necessary, for important elements may be overlooked. At the Mayo Clinic<sup>15</sup> the chances of finding an immature growth in a cervical polyp are about 300:1. Even then the tumor is far more likely to be carcinoma.

The disease is invariably fatal. I know of no authenticated case of recovery. Ordinarily death occurs between one and two years after onset. The case of Bäcker and Minnich<sup>16</sup> is quite unusual in that the patient survived for seven years.

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13. Kunert, E.: *Arch. f. Gynäk.* **6**:111, 1874.

14. Heddäus, A.: *Arch. f. klin. Chir.* **94**:117, 1911.

15. Day, L. A.: *Proc. Staff Meet., Mayo Clin.* **14**:650, 1939.

16. Bäcker, J., and Minnich, K.: *Beitr. z. Geburtsh. u. Gynäk.* **10**:532, 1906.

Even wide surgical excision of the entire genital tract, with radiotherapy added, has yet to cure a patient. Unfortunately, no patient in an early stage has been treated surgically or by radiotherapy, hence no statement can be made as to the curability of the tumor in its early stages.

#### REPORT OF A CASE

A 14 year old white girl was admitted to the Sacramento County Hospital, Sacramento, Calif., Jan. 19, 1940, complaining of a yellowish, bloody vaginal discharge which had been present for six months. The menses had not been affected and occurred every twenty-eight days. The discharge was usually increased after the period. Three weeks prior to admission she passed what she called blood clots. There had been no pain or burning or any other complaint, the patient always having been robust and athletic.

Her parents were living and well; there were no siblings; there was no history of familial disease except carcinoma. The paternal great grandmother died of carcinoma of the breast; both grandmothers died of carcinoma of the stomach.

She was an obese girl, weighing 170 pounds (77 Kg.). She appeared of the stated age and was both alert and cooperative. Whereas elsewhere nothing of note was found, the small vaginal introitus showed multiple protruding grapelike bodies, many of them loose, and free oozing of blood. The entire cervix seemed to have been destroyed with what appeared to be an infiltrating neoplasm. Only after much difficulty was the cervical canal found. Routine laboratory tests, including a negative Aschheim-Zondek test, were noncontributory. The grape-like objects were found solid, of jelly-like consistency and grayish, hyaline in appearance. Dr. J. H. Schaefer reported that the polypoid bodies cut smoothly, leaving a clear firm surface with some submucous hemorrhage. The more superficial polyps showed myxomatous tissue covered with a single layer of squamous epithelium. Although some areas looked quite cellular, the tissue appeared to be essentially that of a mature fibrous polyp. The true nature of the growth became evident, however, on study of tissue taken from the cervix. This tissue was also examined by Dr. Stuart Lippincott,<sup>17</sup> of the National Cancer Institute, and in summary, the tumor revealed highly cellular areas of round and spindle cells, mesenchymal stroma separated by both edema and myxomatous tissue, and striated muscle cells. In addition, and of considerable importance, was the presence of acini, some of which appeared to have actively proliferating cells with heavily chromatic nuclei but which as yet were not truly anaplastic. It was Dr. Lippincott's opinion that the growth was teratoma.

The patient received 1,800 milligram hours of radium, and roentgen therapy was commenced. Six months later (June 1940) there was no local evidence of the tumor, but a hard mass filling the lower half of the abdomen had appeared and the patient had lost some weight. No pulmonary metastases were seen on roentgen examination. Further roentgen therapy caused good regression of the abdominal mass. By September 1940, the vagina was filled with necrotic tumor tissue, with complete infiltration of the right broad ligament, and the vaginal wall was invaded. In November 1940, after the removal of many small jelly-like masses, further tissue was obtained from the cervix. This tissue was grayish white and about the consistency of brain tissue. Part appeared gelatinous. Microscopically there were seen spindle cells in solid masses with clear cytoplasm

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17. Lippincott, S. W.: Personal communication to the author.

and vesicular nuclei. There were frequent irregular mitotic figures. There was great vascularity, and in some areas there was a tendency to the formation of giant cells. There was one small area of early cartilage formation, with a hyaline background and a few cartilage cells.

The patient rapidly declined and died on Dec. 22, 1940, approximately one and a half years after the onset of symptoms. Permission for an autopsy was not obtained.

#### SUMMARY

The nomenclature used for the gross specimen should be employed in designating this tumor pending more exact classification, for there is much yet unknown about the tumor. Pathologists simply do not know why cartilage or striated muscle should appear in the cervix, and the phenomena outlined by Wilms, although attractive, have never been actually demonstrated. The possibility that this type of growth is teratomatous should be kept in mind. The polypoid character appears to depend on the presence of a free cavity, such as the vagina or the pleural cavity. Although no age is exempt, the majority of cases appear between puberty and the menopause. The prognosis is unfavorable.

# PATHOLOGIC CHANGES IN THE LIVER AND KIDNEYS OF GUINEA PIGS DEFICIENT IN VITAMIN C

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During the course of an experiment in which trypan blue was injected into scorbutic guinea pigs, routine sections of kidney and liver were observed to contain greater amounts of the dye than similarly prepared sections from the control animals. This observation was so constant and striking in over 15 animals that another experiment more adequately controlled for this particular finding was undertaken. The following communication reports the results of the latter experiment.

## METHODS

Scurvy was produced in the guinea pigs by feeding a ration known as rabbit chow checkers.<sup>1</sup> When guinea pigs are fed this diet exclusively, characteristic signs of scurvy develop, and the animals die in from twenty-one to twenty-five days. A group of control animals were fed the same diet and, as a source of vitamin C, given lettuce ad libitum. The inanition effect produced by the scurvy was controlled by a second control group of animals, which were given lettuce ad libitum but no rabbit chow, the animals being selectively starved so that they lost the same amount of weight as those receiving the diet deficient in vitamin C. That the disease produced by feeding the rabbit chow checkers was due entirely to the lack of vitamin C was demonstrated by placing a group of young growing guinea pigs (150 Gm.) on this diet and giving them daily intraperitoneal injections of ascorbic acid. The animals so treated maintained normal growth curves and showed none of the signs of vitamin C deficiency noted in the other animals. Several young guinea pigs were included in the experiment to see if age was a factor in the problem. The trypan blue was given subcutaneously in the flank of the animal in a 1 per cent concentration in physiologic solution of sodium chloride. The first injection of the dye was given on the fourteenth day of the experiment, for at this time the animals on the C-deficient diets began to show the first symptoms of scurvy. The amount of the dye given in each instance was

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From the Department of Pathology of the Washington University School of Medicine.

1. The rabbit chow checkers are prepared and sold by the Purina Mills Company, of St. Louis. The rabbit chow is made from soy bean oil meal, wheat germ, corn germ meal, alfalfa meal, ground oats, corn meal, gray wheat middlings,

*(Footnote continued on next page)*

determined by the size of the animal. The adult guinea pigs, weighing from 400 to 600 Gm., were given 50 mg. of the dye on alternate days until a total of 200 mg. was reached. Only 80 mg. of the dye was given to the young guinea pigs weighing from 150 to 200 Gm. All of the animals in a group, with the corresponding control animals, were killed after the first spontaneous death occurred in a scorbutic animal of that group. This period varied from one to four days following the last injection of the trypan blue. Blocks of liver, spleen, kidney, adrenal gland, lung, intestine and heart muscle were fixed in a 3 per cent solution of formaldehyde, and paraffin sections were prepared and stained lightly with trinitrophenol (picric acid). The trypan blue was well preserved with this type of fixation and staining, and was easily seen in the sections.

## RESULTS

The sections of liver and kidney from the scorbutic animals contained remarkably more trypan blue than the sections from the corresponding control animals. There was no significant difference in the amount of the dye deposited in the other organs examined. The livers of the scorbutic guinea pigs showed moderate to advanced fatty degeneration of the liver cells, particularly in the region of the central veins. The vacuolation of the liver cells was definitely identified as a fat by staining frozen sections of liver with sudan III. This fatty change was so constant and characteristic that in sections stained with hematoxylin and eosin those from the C-deficient animals were easily selected from among those representing the control animals. The dye was observed as minute granules indiscriminately placed throughout the cytoplasm of the liver cells, and the cells showing the fatty change stored the greatest amount of dye (fig. 1). In the livers from

molasses, calcium carbonate, iodized salt, and riboflavine concentrate. The exact percentage of each constituent is secret and is not available. The mixture is pressed into small pellets.

Chemical analysis of rabbit chow checkers shows that their composition is as follows:

Protein .....	17.50 per cent
Fat .....	3.60 per cent
Fiber .....	15.00 per cent
Ash .....	6.00 per cent
Nitrogen free extract.....	47.00 per cent
Moisture.....	10.00 per cent
Calcium .....	1.10 per cent
Phosphorus .....	0.42 per cent
Magnesium .....	0.18 per cent
Potassium .....	0.90 per cent
Soluble chloride as Na Cl.....	0.90 per cent
Iron .....	2.75 parts per million
Copper .....	12.00 parts per million
Cobalt .....	0.05 parts per million
Manganese .....	100.00 parts per million
Carotene .....	3.00 parts per million
Vitamin D .....	2 U. S. P. units per gram.
Ascorbic acid .....	0.00 parts per million

The laboratory department of the Purina Mills Company has been unable to identify any ascorbic acid by chemical analysis.

the control animals only a rare granule of dye could be found within the liver cells. The Kupffer cells were filled with the dye both in the scorbutic and in the control animals.

The dye was observed in the cytoplasm of the cells of the proximal convoluted tubules of the kidneys in large granular masses. All of the animals showed significant amounts of the dye, with the scorbutic animals containing unmistakably larger amounts (fig. 2). In the scorbutic animals the dye in many instances so heavily infiltrated a cell that the outline of the nucleus was completely obliterated. Only occasionally did some of the cells of the excretory tubules contain a small amount of dye. No morphologic change was observed in the kidneys to account

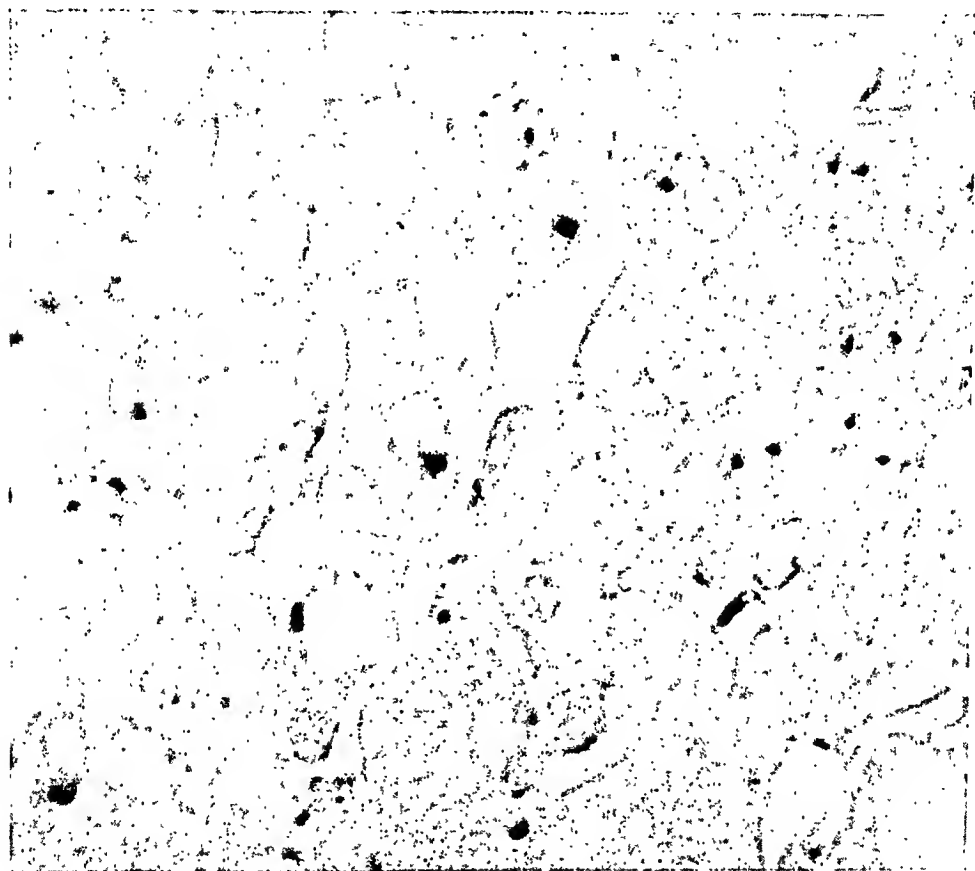


Fig. 1 (C-deficient animal 4).—Section of liver showing the trypan blue as fine black dots within the cytoplasm of the liver cells. Two Kupffer cells, plainly visible in the upper central part of the field, also contain granules of the dye. Note the large vacuoles of fat in the liver cells. Paraffin section with a light trinitrophenol stain;  $\times 2,200$ .

for the increased storage of dye in sections stained with hematoxylin and eosin. Frozen sections of the kidneys stained for fat with sudan III showed no difference in the amount of fat in the deficient and in the control animals. The individual results for the animals in the several groups are recorded in tables 1 and 2. In estimating the amount of dye deposited or the degree of fatty metamorphosis in the liver cells a system of pluses was used. Four pluses indicate the maximal amount of dye or fat observed in any section.

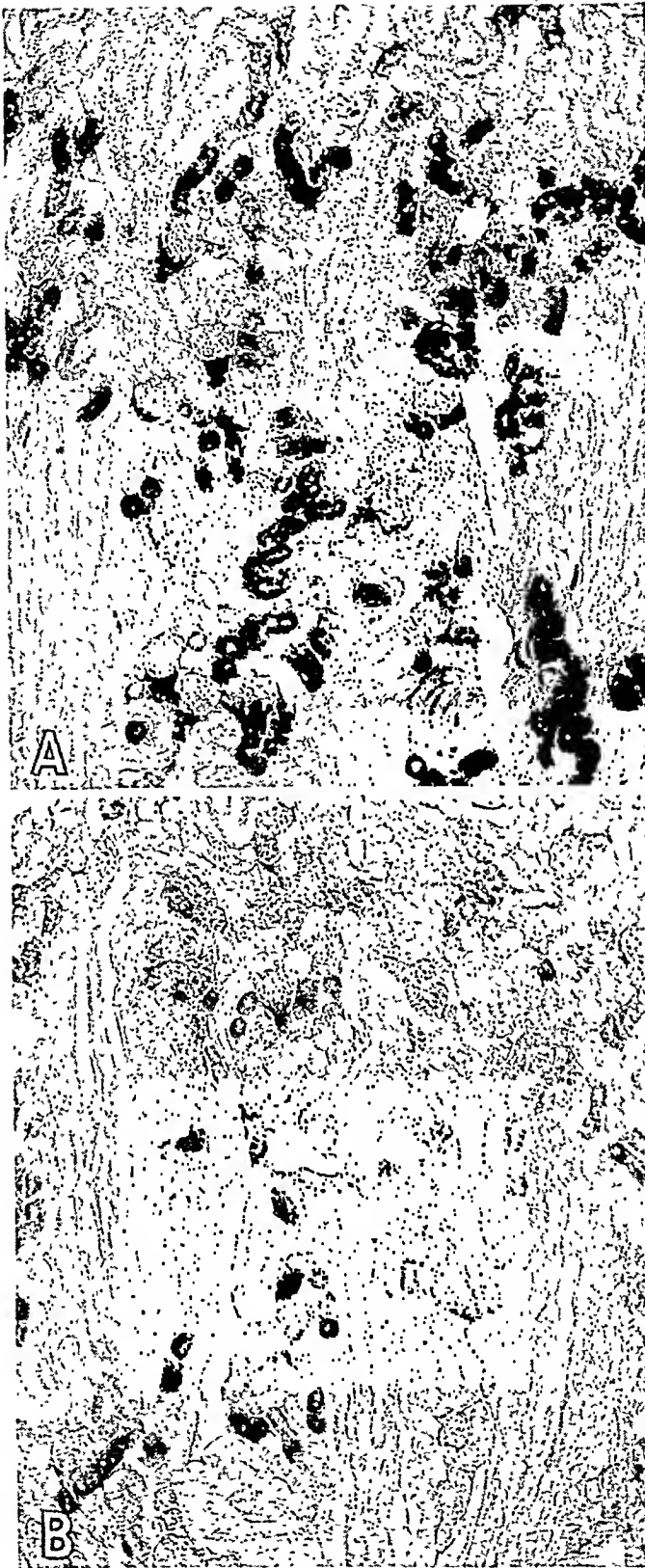


Fig. 2.—*A* (C-deficient animal 5), section of renal cortex showing large granular masses of trypan blue in the cells of the proximal convoluted tubules, completely obliterating all cellular detail. Paraffin section stained lightly with trinitrophenol;  $\times 50$ . *B* (control animal 5), section of renal cortex showing slight amounts of trypan blue in the cells of the proximal convoluted tubules. Compare with *A*. *A* and *B* were photographed and processed under identical conditions. Paraffin section stained lightly with trinitrophenol;  $\times 50$ .



TABLE 1.—*Trypan Blue Deposited in the Liver*

Animal	Guinea Pigs Deficient in Vitamin C				Control Animals			
	Weight, Gm.	Dose of Dye, Mg.	Amount of Dye Deposited	Fatty Meta-morphosis	Weight of Animal, Gm.	Amount of Dye, Mg.	Amount of Dye Deposited	Fatty Meta-morphosis
1	500	200	++++	+++	*508	200	+	+
2	544	200	+++	++++	*527	200	++	0
3	600	200	+++	++	*520	200	+	0
4	591	200	++++	++++	*600	200	+	+
5	568	200	++++	+++	*601	200	+	+
6	540	200	++	++	*552	200	0	+
7	618	200	+++	+++	*588	200	+	0
8	486	200	+++	++	*550	200	+	+
9	590	200	++++	++++	*612	200	+	+
10	525	Died after 1st injection of dye			*557	200	+	0
11	517	Died on 10th day—probably not vitamin C deficiency			*481	200	+	0
12	140	80	+	++++	*203	80	0	0
13	150	80	+	+++	*285	80	0	0
14	296	200	+++	++++	†427	200	+	++
15	377	200	+++	+++	†470	200	+	++
16	146	80	++	+++	†288	80	+	+
17	510	200	++++	++++	†384	200	+	+
18	555	200	+++	++	†307	200	++	+
19	568	200	++++	++++	†382	200	+	+
20	478	Died after 1st injection of dye			†518	200	+	++

\* Received normal control diet.

† Received inanition control diet.

TABLE 2.—*Trypan Blue Deposited in the Kidneys*

Animal	Guinea Pigs Deficient in Vitamin C			Control Animals		
	Weight, Gm.	Dose of Dye, Mg.	Amount of Dye Deposited	Weight of Animal, Gm.	Amount of Dye, Mg.	Amount of Dye Deposited
1	500	200	++++	*508	200	++
2	544	200	+++	*527	200	++
3	600	200	++++	*529	200	+
4	591	200	+++	*600	200	+
5	568	200	++++	*601	200	+
6	540	200	+++	*552	200	++
7	618	200	++++	*588	200	+
8	486	200	++	*550	200	++
9	590	200	+++	*612	200	+
10	525	Died after 1st injection		*557	200	++
11	517	Died on 10th day—probably not vitamin C deficiency		*481	200	++
12	140	80	++	*203	80	+
13	150	80	++	*285	80	+
14	296	200	+++	†427	200	+
15	377	200	++++	†470	200	+
16	146	80	++	†288	80	+
17	510	200	++++	†384	200	+
18	555	200	++++	†397	200	++
19	568	200	++++	†382	200	+
20	478	Died after 1st injection		†518	200	+

\* Received normal control diet.

† Received inanition control diet.

## COMMENT

Because trypan blue has a strong affinity for all pathologically altered cells and dead tissue it appears that the results of our experiment indicate that vitamin C deficiency produces a pathologic change in the cells of the liver and the kidneys of the guinea pig. Since examination of the kidneys from the deficient animals failed to demonstrate any morphologic change, it can only be assumed that the cells of the proximal convoluted tubules where the dye was so heavily deposited were in some way physiologically altered. Further investigation will be needed to understand fully the significance of this isolated fact concerning the kidney in vitamin C deficiency because none of the known effects of vitamin C deficiency to our knowledge is in any way dependent on an altered physiologic function of the kidney.

Aschoff and Koch<sup>2</sup> described advanced fatty degeneration of the liver cells in human cases of scurvy and regarded the change as characteristic of that disease. This observation cannot be regarded as conclusive evidence that vitamin C deficiency in man produces fatty degeneration of the liver, because a spontaneously occurring deficiency disease is usually accompanied by other dietary deficiencies. However, Bessey, Menten and King<sup>3</sup> have showed that a deficiency of vitamin C will produce fatty metamorphosis of the liver in guinea pigs, and our experiment confirms this observation. The fatty change plus the fact that the liver cells in the C-deficient animals contained more trypan blue is good evidence that the vitamin C deficiency had produced a pathologic change in that organ. These findings are particularly interesting in the light of the recent work of Sealock and Silberstein<sup>4</sup> demonstrating that alkaptonuria follows the administration of l-tyrosine to guinea pigs. The severity of the alkaptonuria was observed to be closely correlated with the amount of vitamin C given the animals. Subsequent withdrawal of this vitamin resulted in the reappearance of the homogentisic acid commensurate with the degree of withdrawal. Further evidence that vitamin C is concerned with the metabolism of the aromatic amino acids has been reported by Levine, Gordon and Marples.<sup>5</sup> These investigators observed a spontaneous defect in the metabolism of aromatic amino acids in infants fed cow's milk containing 5 Gm. or more of protein per day. The defect was manifest in the excretion in the urine of 1-p-hydroxyphenylacetic acid and p-hydroxyphenylpyruvic acids.

2. Aschoff, L., and Koch, W.: *Skorbut, eine pathologisch-anatomische Studie*, Jena, Gustav Fisher, 1919, p. 51.

3. Bessey, O. A.; Menten, M. L., and King, C. G.: *Proc. Soc. Exper. Biol. & Med.* **31**:455, 1933.

4. Sealock, R. R., and Silberstein, H. E.: *Science* **90**:517, 1939.

5. Levine, S. Z.; Gordon, H. H., and Marples, E.: *J. Clin. Investigation* **20**:209, 1941.

The administration of ascorbic acid completely eradicated this defect while other vitamin principles were ineffectual. These observations seem to offer good presumptive evidence that vitamin C is concerned with the catabolism of the aromatic amino acids. Since the liver is believed to play an important part in the metabolism of amino acids, the pathologic change in the liver cells as shown in our experiments is morphologic evidence in support of this idea.

#### SUMMARY

Trypan blue injected subcutaneously into scorbutic guinea pigs is more heavily deposited in the parenchymal cells of the liver and in the proximal convoluted tubules of the kidney than in the corresponding cells in control animals receiving the same amount of dye. This observation is interpreted as indicating a pathologic change in these cells as a result of the vitamin C deficiency. In the livers of the scorbutic animals there was a fatty metamorphosis of the liver cells, but no morphologic change was observed in the kidneys to account for the increased deposition of the dye. The morphologic change plus the pathologic deposit of trypan blue in the liver is regarded as evidence of hepatic damage from vitamin C deficiency and as a plausible explanation for the altered aromatic amino acid metabolism accompanying vitamin C deficiency which has been reported by other investigators.

# FIBROUS PLEURAL ADHESIONS

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ST. LOUIS

Fibrous pleural adhesions have been seen so frequently by pathologists that they have occasioned attention only when associated with some obviously causal condition. Previous discussions of pleural adhesions have been concerned with the relation of the lesions to some specific disease or to some definite therapeutic problem. The purpose of this report is to present statistical information regarding the incidence of fibrous pleural adhesions and to analyze the data in terms of possible etiologic agents and factors.

## METHODS

The material for this study was obtained at 400 unselected autopsies on persons whose ages ranged from 1 to 89 years. All these persons were from the population of general hospitals except 66, whose deaths were either traumatic or unexpected. Each specimen was studied without the aid of roentgenograms for anatomic lesions possibly related to fibrous adhesions of the pleura. Microscopic sections of representative adhesions were prepared in approximately one fourth of the cases. Sections of uninvolved pleura were prepared and studied as controls. The microscopic sections were stained with hematoxylin and eosin and by Verhoeff's method for the demonstration of elastic tissue.

## GENERAL INCIDENCE

Of the 400 unselected cases studied at autopsy there were fibrous pleural adhesions in 264, or 66 per cent. It was evident that the incidence of adhesions increased with increasing age. Adhesions were rare in children, but were present in 79 per cent of persons aged 50 (chart). There were no adhesions of the pleura in 50 newly born infants observed at autopsy in this department but not included in this series. With regard to the persons less than 20 years of age, the apparent causes of the adhesions were found at autopsy in isolated instances. The associated causal conditions included unresolved pneumonia, bronchiectasis, abscesses of the lungs, empyema and rheumatic fever. In older persons the causes were usually not apparent.

## INCIDENCE IN RELATION TO PARTICULAR CONDITIONS

*Past Pneumonia.*—The frequent association of pneumonia and fibrous pleural adhesions in younger persons suggested a causal rela-

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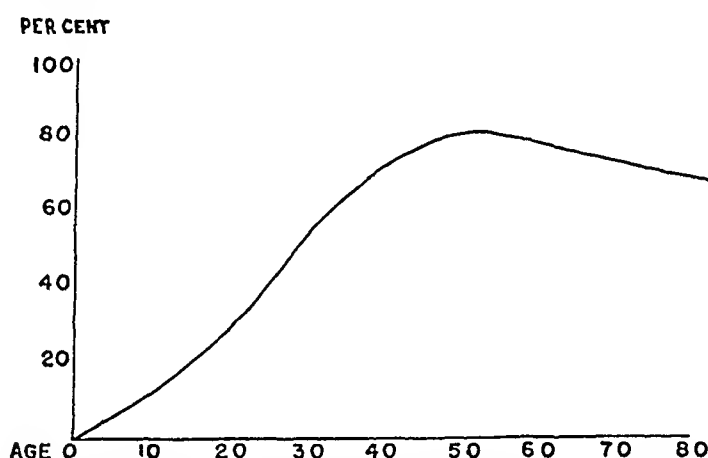
From the Department of Pathology of Washington University School of Medicine.

tion. Past histories of pneumonia or pleurisy or both were obtained from the charts of 49 patients. Forty-six of these patients, or 94 per cent, showed fibrous adhesions of the pleura at autopsy. This figure was significantly high, and all ages were represented in the group.

*Calcified Nodules and Apical Scars.*—The entire series was divided into five groups (table 1).

The first group, 97 patients, had no history of pneumonia and had no evidence of tuberculosis or other chronic inflammation. Only 50, or 52 per cent, showed fibrous pleural adhesions.

In the second group were 125 whose lungs contained calcified nodules. The nodules were interpreted as latent lesions of the first infection type of tuberculosis. There were no lesions of the reinfection type. Seventy specimens, or 56 per cent, showed adhesions which only occasionally were in the vicinity of a calcified nodule.



Percentage incidence of fibrous pleural adhesions at various ages as determined on specimens obtained post mortem from 400 persons.

TABLE 1.—Incidence of Fibrous Pleural Adhesions

Group	Patients	Average Age	Incidence of Adhesions, %*
No pneumonia and no tuberculousis.....	97	41	52
Calcified pulmonary nodules.....	125	47	56
Apical scars .....	125	59	81
Past pneumonia or pleurisy or both.....	49	50	94
Chronic pulmonary inflammation.....	4	..	100

\* The incidence increases with the average age of the group and is not necessarily related to the lesions.

The third group included 125 patients from whom specimens were obtained having one or two apical scars in addition to calcified nodules. The apical scars were considered to be latent lesions of the reinfection type of tuberculosis. In 101, or 81 per cent, of these the pleura showed fibrous adhesions, a few of which were located over the scars.

The fourth group consisted of 49 patients with a past history of pneumonia or pleurisy or both. Ninety-four per cent showed adhesions.

The fifth group included 4 patients whose lungs presented chronic inflammation; one pair of lungs showed advanced tuberculosis, and three pairs showed advanced bronchiectasis. The adhesions were obviously related to the areas of inflammation.

The high incidence of fibrous pleural adhesions in the last two groups was significant. In the first three groups the incidence appeared to be increased in those with tuberculous lesions of the first infection and reinfection types. However, analysis showed that the incidence of pleural adhesions in the first three groups (table) increased with the average ages of the groups. Therefore, these figures showed no relation between latent tuberculosis and fibrous pleural adhesions.

*Apical Scars and Apical Adhesions.*—The pleura over apical scars was not the commonest site for the localization of fibrous pleural adhesions. In 125 lungs, totaling 250 apexes, there were 209 apical scars. Adhesions were associated with 33 of the 209 scars, or 16 per cent. Conversely, there were adhesions over 79 of the 800 apexes in 400 pairs of lungs. Beneath 27 of the 79 adhesions there were apical scars. From these figures it is evident that about 1 apical scar in 6 had an overlying adhesion and that only 1 apical adhesion in 3 was associated with an apical scar.

#### AREA OF PLEURA INVOLVED

In each of the 400 specimens the area of pleura covered by adhesions was estimated. Forty-six persons with a history of pneumonia or pleurisy or both showed an average area of involvement of 23 per cent of the total pleural surface. One hundred and one persons with apical scars had adhesions covering an average area of 9 per cent of the pleura. Sixty-nine persons with calcified nodules had an average pleural area of 8 per cent involved. Fifty-two persons with no nodules, scars or other chronic lesions had an average area of 10 per cent of the pleura involved. The average surface area of pleura covered by adhesions in the pneumonia-pleurisy group was at least twice the average surface area covered in any and all other groups.

#### TYPE AND LOCATION OF ADHESIONS

The fibrous pleural adhesions in the 400 unselected autopsy subjects were classified as (1) diffusely arranged or (2) isolated strands. Four general locations were arbitrarily chosen as (1) anterior, (2) posterior, (3) upper and (4) lower parts of the pleura (table 2). Many pleurae showed both diffuse and isolated types of adhesions, and a majority of the specimens showed adhesions in more than one location.

In the group with past histories of pneumonia or pleurisy or both there were isolated adhesions in 57 per cent and diffuse adhesions in 59 per cent. It was striking that in the remaining groups the incidence of diffuse adhesions was low. This suggested that the adhesions in the pneumonia group tended to be diffuse and that the incidence of isolated adhesions in this group was relatively low because the diffuse adhesions obscured the isolated ones.

From the data in table 2 it is evident that most of the pleural adhesions were located over the posterior aspect of the lungs. In the pneumonia group the extensiveness of the diffuse adhesions served to increase the incidence in all locations. However, the predominance of adhesions in the posterior location was maintained throughout. The anterior location was a less frequent site. The lower parts of the pleura were the sites of fibrous adhesions much more frequently than the upper

TABLE 2.—*Types and Locations of Fibrous Pleural Adhesions\**

Type of adhesion:	Group with Pneumonia, %	Group with Apical Scars, %	Group with Calcified Pulmonary Nodules, %	Group with No Tuber- culosis or Pneumonia, %
Isolated.....	57	76	81	73
Diffuse.....	59	27	29	35
Location:				
Posterior.....	85	65	69	67
Anterior.....	69	42	56	65
Lower.....	49	35	23	38
Upper.....	26	28	20	12

\* The most common fibrous pleural adhesion is an isolated one occurring over a lower lobe posteriorly.

parts, which include the apexes. From these findings it is evident that the most common fibrous pleural adhesion in this series was an isolated one occurring posteriorly over a lower lobe.

#### MICROSCOPIC TYPES

The great majority of the adhesions observed were delicate fibrous structures which were additions to the pleura and were not associated with permanent changes in the parenchyma beneath. There was slight subpleural thickening beneath and around the typical fibrous pleural adhesion. The thickening was composed of fibrous tissue between the pleura and the definite subpleural elastic layer. The thickening was most prominent beneath the point of adhesion and gradually became thinner away from the point. As a rule, isolated and diffuse adhesions did not differ except in extent.

A few of the adhesions disrupted the subpleural elastic layer and were composed of abundantly vascularized fibrous tissue. Lesions of this type did not constitute a significant percentage of the adhesions in the specimens observed.

## COMMENT

That fibrous pleural adhesions are associated with inflammations of the lungs is supported by the statistical evidence presented in the foregoing paragraphs. Pneumonia is the most common and outstanding related disease. Persons who have had pneumonia or pleurisy or both have a high incidence of adhesions that are extensive. Further, the locations of the adhesions in persons who have had pneumonia correspond to the more common sites of pneumonia; i. e., they are to be found posteriorly on the lower lobes. Fibrous adhesions are not congenital. Newly born infants do not have them, and the incidence increases as time permits the development of associated conditions. Lungs with focal inflammation frequently present evidence that inflammation is related to adhesions. This is most convincing in a young person showing isolated fibrous strands over an area involved by a solitary abscess, over a lingula of the lung showing bronchiectasis or only in the vicinity of a tuberculous cavity. Infarcts of the lung may have associated overlying adhesions.<sup>1</sup> The inflammation of the pleura over the infarct probably is the principal causal factor.

Most fibrous pleural adhesions are without historical or anatomic evidence to suggest a related disease or condition. Perhaps the cause is not manifest clinically; the patient may forget the incident; the doctor may obtain an incomplete past history; the methods of examination may be inadequate, or the predisposing condition may leave no permanent change except the adhesions.

Masses and nodules of a cancer are related to fibrous pleural adhesions only when inflammation occurs with them. Carcinoma of the bronchus frequently causes obstruction of the bronchial lumen with subsequent atelectasis and pneumonia. Adhesions form over the inflamed portion of the lung and not in the vicinity of the obstructing mass of tumor. Metastatic nodules of tumor from remote organs do not commonly predispose to pneumonia and therefore are not associated with adhesions. Strands of tumor tissue in subpleural lymphatic channels do not produce an appreciable inflammatory reaction.

Although tuberculosis is an inflammatory disease, there is scant evidence that subclinical and latent lesions are associated with fibrous pleural adhesions. The adhesions over the lungs of the group with calcified nodules, with or without scars of the apexes, are not significantly different in incidence, extent, location and type from those in a similar age group without such lesions. Only occasionally an isolated fibrous adhesion is located over a calcified nodule. Of the apical scars having overlying fibrous adhesions there are some which are convincing in their relation. Microscopically, these few adhesions are dense fibrous

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1. Castleman, B.: *Arch. Path.* **30**:130, 1940.



strands that are continuous with the fibrous tissue of the apical scars. Blood vessels with muscular walls are continuous from the scars through the adhesions to the parietal pleura. The subpleural elastic and fibrous layers are interrupted, although the scar contains persistent elastic tissue which preserves the outlines of the obliterated alveoli.

From the observations on a few lungs showing radiation pneumonitis there is no evidence that fibrous pleural adhesions are directly a result of roentgen ray irradiation. Warren and Gates,<sup>2</sup> from experimental and pathologic observations, stated that "extensive fibrosis and pleural adhesions may be ascribed to inflammation or infection, intercurrent or resulting from the radiation-induced changes." Their view is in accord with the idea that inflammation is the basic cause of fibrous pleural adhesions.

Patients giving either historical or anatomic evidence of rheumatic fever show no increase in the incidence of fibrous pleural adhesions. Likewise, patients with advanced mitral stenosis and fibrous pericardial adhesions do not show an increase in incidence of adhesions of the pleura. However, a few specimens from persons with active rheumatic heart disease showed adhesions of the pleura limited to the region of the pericardium. From these observations it appears that in an occasional case active rheumatic heart disease is accompanied by fibrous pleural adhesions near the heart.

Arteriosclerosis and arteriolosclerosis of the pulmonary arteries, observed grossly and microscopically, do not show a relation to adhesions of the pleura. It is true that blood vessels show sclerosis when they course in scars of the apexes, around chronically inflamed lesions of bronchiectasis, near abscesses and about tuberculous cavities. However, this alteration may be considered a result of the inflammation. The only suggestion of an association is that single isolated adhesions are attached over interlobular septums in numerous instances, and the only accompanying lesion is sclerosis of the vessels in the septum.

One group of 35 specimens was from persons killed accidentally and without preceding manifest disease. A comparison of this group with the specimens from persons with chronic diseases showed no significant difference in the incidence of fibrous pleural adhesions.

The specimens from patients with manifest diseases were divided into groups including (1) those with chronic cardiac disease, (2) those with cancer, (3) those with chronic infections and (4) those with acute infections. The curve of incidence of fibrous pleural adhesions in these groups corresponded to the curve of the average age of the persons in the groups. Otherwise there were no significant differences in incidence or in location of the adhesions.

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2. Warren, S., and Gates, O.: *Arch. Path.* **30**:440, 1940.

Besides the several conditions already discussed, other possible factors were studied. The following could not be shown to be of significance: race, sex, increased blood pressure, inhalation anesthesia, emphysema of the lungs, anthracosis of the lungs, duration of primary disease, cause of death and economic status of the subject.

#### SUMMARY

Sixty-six per cent of the pairs of lungs obtained at 400 unselected autopsies showed fibrous pleural adhesions. The incidence of fibrous pleural adhesions increased with age. Of 49 patients giving a past history of pneumonia or pleurisy or both, 94 per cent had fibrous pleural adhesions, which were twice as extensive as the adhesions in groups without a history of pneumonia. The most common pleural adhesions were isolated ones occurring over the lower lobes posteriorly. One sixth of the apical scars were associated with fibrous pleural adhesions. Two thirds of the apical adhesions were not over scarred apexes. The great majority of fibrous pleural adhesions were over essentially normal pulmonary tissue.

# FORSSMAN'S "CAROTID SYNDROME"

A CONTRIBUTION TO THE STUDY OF ANAPHYLACTIC CHANGES  
IN THE NERVOUS SYSTEM FROM THE STANDPOINT  
OF PATHOLOGY

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THIELS, N. Y.

The term "carotid syndrome" is used by Forssman<sup>1</sup> to denote neurologic disturbances occurring in the guinea pig when a small dose of serum containing Forssman antibodies is injected into the carotid artery. This syndrome consists of disequilibrium, rotary movements along the vertical and the longitudinal axis, forced deviation of the eyeballs and nystagmus. Manifestations of anaphylactic shock which are commonly observed when Forssman serum is injected intravenously are absent or scanty. Under these experimental conditions the serum, which is slowly injected centripetally into the right carotid artery, reaches through the subclavian and vertebral arteries, the hindbrain and the midbrain, where a reaction presumably takes place between Forssman antibodies in the injected serum and Forssman antigen normally present in the nervous tissue. That Forssman antibodies are responsible for the syndrome is apparent from the fact that no clinical manifestations occur when these antibodies are removed from the serum either by absorption with guinea pig kidney or by heating at 80 C. However, a similar syndrome is produced by injecting large amounts of normal rabbit, ox or eel serum, cobra venom (Friedberger and Oshikawa<sup>2</sup>) and suspensions of lyco-podium or starch (Forssman<sup>1c</sup>).

Although the serologic problems concerning this syndrome have been the object of numerous investigations, little has been published on its pathologic aspects. Friedberger and Schröder<sup>3</sup> reported the pathologic changes occurring within forty-eight hours in 8 guinea pigs as studied in the Nissl and hematoxylin-eosin preparations. Lesions of necrotic nature were found which were considered vascular in origin; there were

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From the Research Department of Letchworth Village.

1. Forssman, J.: (a) *Biochem. Ztschr.* **110**:164, 1920; (b) **133**:114, 1922; (c) *Acta path. et microbiol. Scandinav.* **3**:749, 1926.

2. Friedberger, E., and Oshikawa, K.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **33**:48, 1922.

3. Friedberger, E., and Schröder, P.: *Ztschr. f. d. ges. exper. Med.* **26**: 287, 1922.

no hemorrhages, no thromboses and no softenings. Ingvar<sup>4</sup> and Skoog<sup>5</sup> briefly reported the observation of capillary hemorrhages in the medulla oblongata in guinea pigs which had died a few hours after the injection. Finally, Skoog<sup>6</sup> and Broman,<sup>7</sup> using the method of vital staining, found increased permeability of the hematoencephalic barrier in the regions which are reached by the Forssman serum.

In view of this scarcity of pathologic reports, it was considered of interest to study the lesions underlying the "carotid syndrome." It was thought, moreover, that such investigation might offer additional data for the solution of the previously investigated problem of the pathologic aspects of anaphylactic reactions in the central nervous system (Jervis and co-workers<sup>8</sup>).

#### METHODS

Two types of Forssman serum were used. The first was obtained from rabbits given six injections of an aqueous extract of guinea pig kidney over a period of three weeks. The extract was freshly prepared by grinding two kidneys in a mortar with approximately 20 cc. of saline solution; the suspension was centrifuged at low speed, and the supernatant fluid was injected intravenously into the marginal vein of the rabbit's ear. The second type of serum was obtained from rabbits prepared by injecting 10 to 15 cc. of a 5 per cent suspension of fresh sheep red cells twice a week over a period of three weeks. The rabbit serum, obtained one week after the last injection, was inactivated at 60 C. for thirty minutes. Both serums produced typical "reversed" anaphylactic shock when injected intravenously into guinea pigs at doses of 1 to 2 cc.

Doses of 0.1 to 0.3 cc. diluted to 0.5 cc. with physiologic solution of sodium chloride were injected slowly (over eight to ten seconds) into the right carotid artery in guinea pigs in the centripetal direction. The artery was tied distally from the site of injection shortly before the injection and proximally to the site of injection immediately after. Some 50 guinea pigs were given these injections. The animals were killed and autopsies made at various intervals from a few minutes to several days. The central nervous system was fixed either in solution of formaldehyde or in alcohol, and the following stains were used: Nissl's for neuron cells, Spielmeyer and Weil stains for myelin sheaths, Bodian's for axis cylinders, Pickworth's for blood vessels, Wilder and Mallory's for connective tissue, Holzer's, Cajal's and Hortega's for glia and scarlet red for fatty products of degeneration. In a few cases, intravital staining of the central nervous system was obtained by injecting intravenously 10 to 15 cc. of a 1 per cent solution of trypan blue a few minutes after the administration of serum.

In addition, 5 guinea pigs were given 0.3 cc. of Forssman serum subdurally through a trephine opening in the skull. Finally, 10 animals were given intra-carotid injections of a 5 per cent suspension of starch.

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4. Ingvar, S.: *Acta path. et microbiol. Scandinav.* **4**:349, 1927.

5. Skoog, T.: *Acta oto-laryng.*, 1939, supp. 32, p. 1.

6. Skoog, T.: *Acta oto-laryng.* **25**:365, 1937.

7. Broman, T.: *Skandinav. Arch. f. Physiol.* **80**:59, 1938.

8. Jervis, G. A.; Ferraro, A.; Kopeloff, L., and Kopeloff, N.: *Arch. Neurol., & Psychiat.* **45**:733, 1941.

## RESULTS

In the majority of animals which were given the intracarotid injection of Forssman serum the typical "carotid syndrome" occurred. Its clinical manifestations corresponded closely to those described by Forssman<sup>1</sup> and Skoog.<sup>6</sup> Immediately after the injection there was observed forced rotation of the head and spine to the left; the limbs on the right side were in spastic extension and exhibited tremors and clonic movements. When the animal was left free, it showed a tendency to move in a circle counterclockwise. In addition, there was rotation along the longitudinal axis to the left (sinistrotorsion). The eyes showed deviation, the right eye toward the nose and the left one in the opposite direction. Nystagmus was frequently observed. This syndrome lasted from a few minutes to several days. About 50 per cent of the animals died. The other animals at times recovered completely and at other times showed various neurologic disturbances, such as tremors, paralysis of limbs and tilted position of the head.

The "carotid syndrome" was occasionally observed also in the animals which were given suspensions of starch. It was much less constant and less characteristic. No animals of this group died, and in no instance did the symptoms last more than one hour.

Following the subdural injection of Forssman serum, the guinea pigs showed jerking movements, repeated convulsions and coma; all these animals died within twenty-four hours.

For a description of the pathologic observations, the animals showing the typical "carotid syndrome" may be divided into three groups according to the periods of time elapsing between the injection of Forssman serum and death.

The first group includes the animals which died or were killed within twelve hours after the injection. It consisted of 18 guinea pigs, 10 of which were fixed in solution of formaldehyde and 8 in alcohol. The pathologic picture in the Pickworth preparation was characteristic (fig. 1). The blood vessels of the right half of the medulla oblongata were dilated. A striking difference of vascular pattern between the two halves of the medulla thus resulted. Several degrees of vasodilatation were seen, which were usually correlated with the intensity of the clinical symptoms. When marked dilatation had taken place, small hemorrhages were observed (fig. 1C). These, however, were seen only in animals which showed severe symptoms followed shortly by death. The vasodilatation often extended to the right half of the pons and, to a lesser extent, to the cerebral pedunculus. The right half of the cerebellum occasionally showed vascular dilatation. No hemorrhages were seen in these regions. The endothelium of the dilated vessels often showed swelling in the hematoxylin-eosin preparations. Edema of the perivascular space was frequently seen, but perivascular infiltration was absent. The nerve cells as seen with the Nissl method showed diffuse alterations in the areas of vascular dilatation. Swelling of the cell and chromatolysis were the most frequently encountered lesions, while vacuolation of the cytoplasm was rarely seen. These lesions were more pronounced in

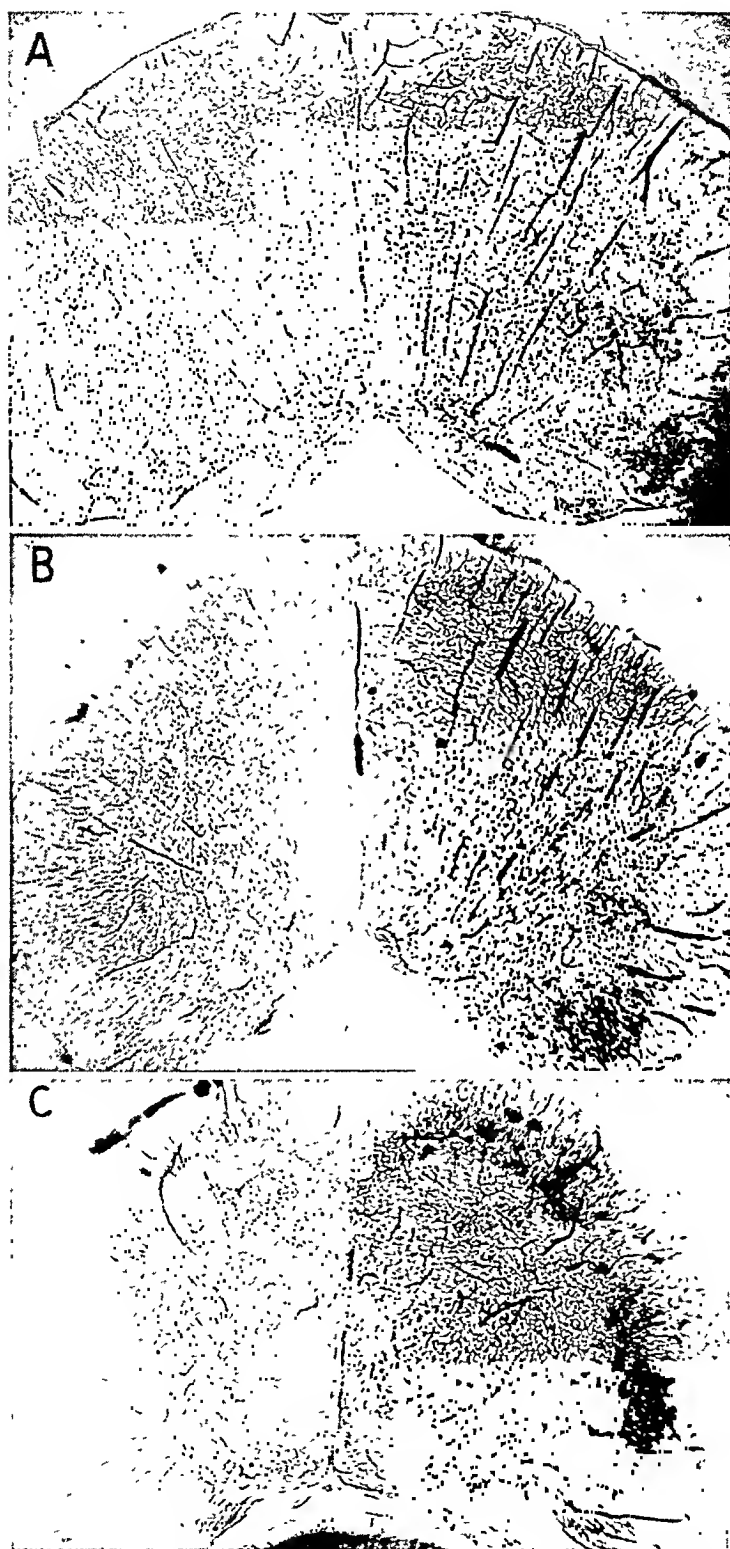


Fig. 1.—Various degrees of vascular dilatation in the right half of the medulla oblongata. Pickworth stain; low power magnification.

the animal that died several hours after the injections. The glia showed no apparent reaction. In the myelin preparation the right half of the medulla was frequently paler than the left half. This appeared to be due to edema which infiltrated among the myelin sheaths more than to actual destruction. However, occasionally some swelling and breaking down of individual sheaths could be observed in the affected areas.

A second group includes the animals which died or were killed between twelve and forty-eight hours after the injection of Forssman serum. It included 9 animals, 4 of which were fixed in solution of formaldehyde and 5 in alcohol. The Pickworth preparation showed vascular dilatation as described in the previous group only in a few instances, those in which death occurred before the eighteenth hour. Later on, the vascular pattern appeared normal with the exception of occasional small hemorrhages. In a few cases in which considerable edema was present in the affected half, the blood vessels appeared smaller than those in the normal side. Diffuse alterations of the nerve cells, more pronounced than in the previous group, were observed. They consisted of swelling of the cell body with chromatolysis and disappearance of the nucleus (fig. 2*A*) or vacuolation and shrinkage of the cytoplasm with nuclear pyknosis (fig. 2*B*). A characteristic lesion appeared in animals of this group from twenty-four to forty-eight hours after the injection. It consisted of small scattered foci of "softening." In the hematoxylin-eosin and the Nissl preparations these foci appeared as small areas of bleaching; the nerve cells had disappeared or were severely degenerated, and only fragmented nuclei and cellular detritus were seen. The glia cells appeared likewise damaged, showing no reactive elements at this stage. In the myelin preparation the foci appeared as demyelinated patches; fragments of myelin sheaths and swollen fibers were seen particularly at the periphery. In the silver preparation the axis-cylinders were partly destroyed. No fatty material was detectable with scarlet red. The foci contained no hemorrhages or hematogenous elements, such as lymphocytes or leukocytes. There was apparently no constant relation to blood vessels; although blood vessels were present within the focus, the lesion was rarely perivascular. These foci were scattered irregularly in the right half of the medulla and pons. Occasional foci were seen in the cerebellar pedunculi and midbrain. They varied in size from very small to large ones, the latter occupying about one eighth of the medulla. The configuration was generally irregular, roundish shapes predominating over elongated ones.

A third group includes 8 guinea pigs which had lived from five to ten days after the injection. In the Pickworth preparation no significant alterations were found. In the Nissl preparations, lesions of the nerve cells such as those seen in other groups were scanty. The characteristic feature of this group consisted of the presence of scattered foci that in distribution, size and shape were similar to the foci described in the previous group. In the myelin preparation (fig. 3) the foci appeared as sharply defined areas of demyelination; the destruction of myelin was complete, and only in a limited peripheral zone were swellings and fragmentations of myelin sheaths discernible. In the silver preparation a considerable number of the axis-cylinders within the lesions were destroyed; the remaining ones showed irregular swellings and fragmentations. With cellular stains (fig. 4) the lesion was seen to be filled with compound granular elements. These in the Nissl and hematoxylin-eosin stains showed large amounts of foamy cytoplasm and small eccentric nuclei; with the Herxheimer stain, the cytoplasm contained characteristic red granules of fat. Occasionally, large compound granular cells containing two nuclei were present, but giant cells were not seen. Hematogenous elements, such as lymphocytes, plasma cells and leukocytes, were scanty.

Macroglia cells were absent within the patch; only at the periphery and in the adjacent normal nervous parenchyma some evidence of macroglial reaction could be observed; hypertrophic and hyperplastic macroglial elements were present

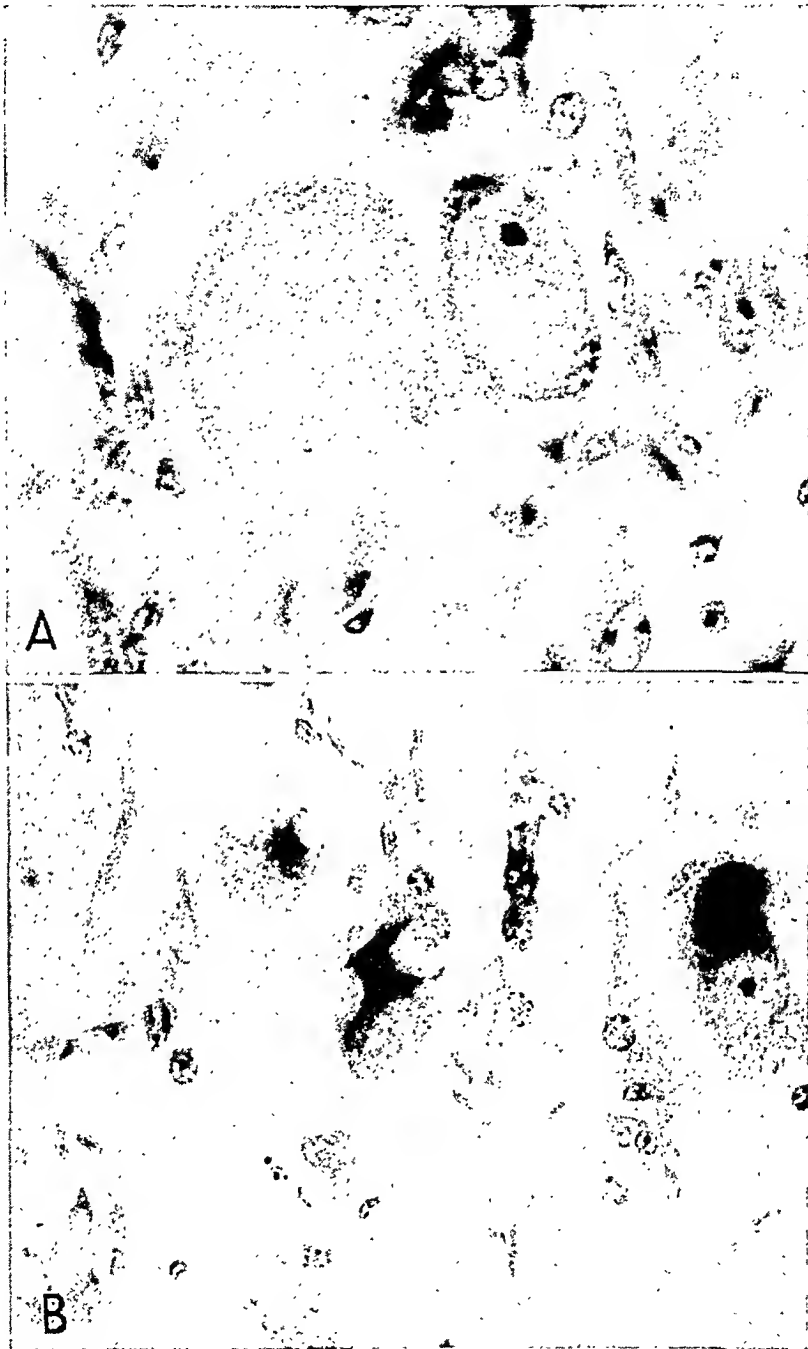


Fig. 2.—Degenerative changes of the nerve cells: *A*, swelling and chromatolysis. *B*, vacuolation, shrinkage and shadow cell. Nissl stain; high power magnification.

here. Occasionally, degenerative types of glia cells were seen. In the Hortega preparation the periphery of the lesion disclosed various phases of transformation



of microglia cells into compound granular elements. The connective tissue stain revealed no connective fibers within the lesion. In the Mallory preparation the blood vessels showed no evidence of thrombosis. There were no hemorrhages. The vessel walls were often thickened owing to proliferation of the intima and adventitia.

In the animals in which vital staining was done, the pathologic picture was very characteristic: The right half of the medulla, pons and cere-

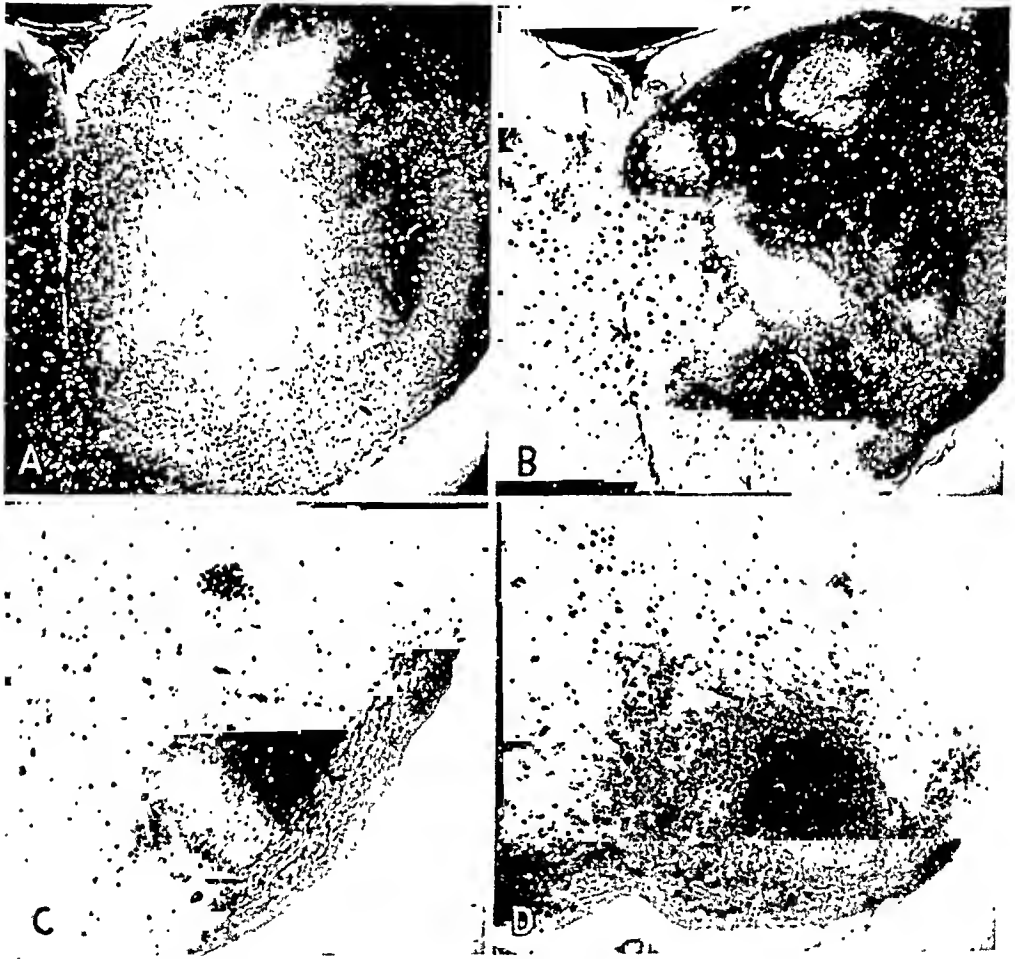


Fig. 3.—Circumscribed foci of demyelination in the medulla oblongata (*A* and *B*), in the cerebral pedunculus (*C*) and in the pons (*D*). Weil stain; low power magnification.

bellum showed an intense blue color, while the other parts of the central nervous system remained unstained.

The animals which had received starch suspension into the carotid artery and which exhibited neurologic disturbances showed mainly vascular changes. In the Pickworth preparations of the medulla the vascular pattern of the right half was altered, showing localized areas of anemia and hyperemia with frequent small hemorrhages. There were



Fig 4.—Foci of demyelination in the Nissl preparation showing mainly compound granular corpuscles. Nissl stain; medium power magnification.

no diffuse changes in the neuron cells, no circumscribed foci of demyelination and no microglial reaction.

The pathologic changes in the animals which received the subdural injection of Forssman serum consisted of pronounced congestion of the meningeal blood vessels and infiltrations of the pia with leukocytes and lymphocytes. Subpial hemorrhages were frequently seen; in 1 instance there were small perivascular hemorrhages in the external cortical layer and in the periventricular regions.

#### COMMENT

The histologic picture of the "carotid syndrome" brought about by Forssman serum is vascular and parenchymatous in character. In the first stage, the blood vessels in the areas reached by the Forssman serum are dilated and congested, endothelial damage is apparent and small hemorrhages may occasionally occur. That the damage to the blood vessels brings about an abnormally increased permeability of the hematoencephalic barrier appears from the results of experiments with vital staining. It is well known that when trypan blue or similar dyes are injected intravenously into a normal animal, no part of the central nervous system retains the dye, a hypothetical barrier between the blood vessel and the brain preventing the coloring substance from passing through the vascular wall into the nervous parenchyma. However, as Skoog<sup>6</sup> and Broman<sup>7</sup> reported, when a dye is given following an intra-carotid injection of Forssman serum, one half of the medulla, pons and cerebellum is markedly stained, thus indicating that a breaking down of the hematoencephalic barrier occurs in the region reached by the Forssman serum.

This "vascular stage" is followed in a certain number of cases by parenchymatous lesions, both diffuse and patchy in distribution, the former being characterized by degenerative changes of the neuron cells and a mild reaction of the glia, the latter by circumscribed foci of softening. The histologic characteristics of the circumscribed foci correspond closely to those described by Hassin<sup>9</sup> in "multiple degenerative softenings." In this condition the softened areas are in the form of multiple irregularly scattered patches of demyelination which do not follow the tracts of nerve fibers and do not depend on territorial blood supply. The blood vessels show neither thrombosis nor embolism. Compound granular corpuscles of microglial origin constitute the content of the softened foci. Fibers of connective tissue are absent, and there is no formation of a scar or a capsule.

The parenchymatous lesions may be explained by assuming that the Forssman antibodies, passing through the impaired hematoencephalic barrier, come in contact with antigen normally present in the tissue of

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9. Hassin, G. B.: *J. Neuropath. & Exper. Neurol.* 1:200, 1942.

the guinea pig. A reaction between antigen and antibody takes place, resulting in damage of the nervous parenchyma. This reaction may be considered of anaphylactic nature, following the widely accepted definition of Doerr<sup>10</sup> that the common character of anaphylactic phenomena is the reaction between antigen and antibody at the cellular site of the antibody or the antigen. Commonly the antibody is present in the cells and the antigen is of exogenous origin, while in the present experiments the antigen is present in the tissue and the antibody is injected. That the reaction of Forssman's antigen and antibody represents a local anaphylactic phenomenon appears to be substantiated by the results of the subdural administration of Forssman's antibodies in guinea pigs. A severe rapid reaction was obtained, characterized by necrosis of the walls of blood vessels, edema and degeneration of connective fibers of the meninges, hemorrhages and infiltration with leukocytes. As is well known, these morphologic features are characteristic of the Arthus phenomenon. Kallos and Kallos-Deffner<sup>11</sup> recently described similar changes in the peritoneum of the guinea pig following the injection of Forssman serum and concluded that these findings represent manifestations of local anaphylaxis.

It appears possible that local anaphylactic reactions occur also in the endothelial cells of the blood vessels, before the antibodies reach the parenchyma. Evidence in favor of this hypothesis explaining the vascular damage referred to in the foregoing section on an anaphylactic basis is offered by the observations of Kallos and Kallos-Deffner<sup>11</sup> and Halber<sup>12</sup> indicating that large amounts of Forssman antigen are present in the vascular endothelium of the "cavia group" of animals.

On the basis of these results the symptoms comprised in the carotid syndrome are easily explained: Unilateral parenchymatous lesions of the vestibular nuclei will result, in fact, in disequilibrium, forced rotary movements and nystagmus. The same symptoms are likely to occur also when vascular changes and reversible cellular alterations take place as in the animals that recovered spontaneously and showed little, if any, parenchymatous alterations. The occurrence of a transitory and often incomplete carotid syndrome following the injection of starch apparently also indicates a vascular mechanism; in this case, however, the damage of the blood vessels and the consequent impairment of the cellular function are brought about by small embolisms independently of any anaphylactic phenomenon.

Of particular interest appear to be the circumscribed lesions of demyelination and microglial reaction found in the animal that showed marked symptoms for a period of more than a week. Similar lesions

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10. Doerr, R.: *Ztschr. f. Hyg. u. Infektionskr.* **118**:623, 1936.

11. Kallos, P., and Kallos-Deffner, L.: *Schweiz. Ztschr. f. allg. Path. u. Bakt.* **5**:97, 1942.

12. Halber, W.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **39**:282, 1924.

have been previously found to occur in the central nervous system of the monkey given extract and emulsion of rabbit brain (Rivers and Schwentker<sup>13</sup>; Ferraro and Jervis<sup>14</sup>); it was then assumed that the pathologic changes were due to brain-specific antibodies, the reacting antigen being a lipoid present in the extract activated by a heterologous protein contained in the emulsion. Moreover, scattered demyelinating lesions of the same type were found in the brains of monkeys in which anaphylactic shocks had been produced in various ways (Jervis and co-workers<sup>8</sup>); the hypothesis was advanced that an antigen-antibody reaction was at the basis of the demyelinating lesion, the brain-specific antibodies being the product of complete antigens formed by the injected foreign protein acting on the damaged brain tissue. The circumscribed lesions of demyelination here described, which apparently are also the result of an antigen-antibody reaction within the central nervous system, show similar histologic characteristics, although hematogenous elements were rarer and giant cells absent. Differences in the properties of the antigen and the antibody and the intensity of the reaction may explain these differences in histologic details.

Evidence has been thus accumulating which indicates that scattered demyelinating lesions of the central nervous system ("multiple degenerative softenings") can be experimentally produced at the site of an antigen-antibody reaction. Such findings may eventually offer some clue in the study of the genesis of certain demyelinating conditions in man, the nature of which is still unknown.

#### SUMMARY

The pathologic changes which underlie Forssman's "carotid syndrome" in the guinea pig are described. This syndrome occurs when Forssman antibodies are injected into the right carotid artery. The right half of the hindbrain and midbrain was found to show marked dilatation of blood vessels and damage of the endothelium, followed by parenchymatous lesions. These consisted of diffuse degenerative changes of nerve cells and circumscribed foci of demyelination with microglial reaction ("multiple degenerative softenings"). The parenchymatous lesions are considered to be anaphylactic in nature, resulting from the reaction of Forssman antibodies which have passed through an impaired hemato-encephalic barrier with Forssman antigens normally present in the tissue of the guinea pig.

From these and similar findings previously reported, it is apparent that scattered demyelinating lesions of the central nervous system ("multiple degenerative softenings") can be experimentally produced at the site of an antigen-antibody reaction.

13. Rivers, T. M., and Schwentker, F. F.: *J. Exper. Med.* **61**:689, 1935.

14. Ferraro, A., and Jervis, G. A.: *Arch. Neurol. & Psychiat.* **43**:195, 1940.

# NEUROBLASTOMA OF THE MEDIASTINUM WITH PHEOCHROMOBLASTOMATOUS ELEMENTS

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Tumors arising from the sympathetic nervous system have been the object of much interest and study. The first case adequately described was that reported by Virchow in 1864 (Wahl<sup>1</sup>; Lewis and Geschickter<sup>2</sup>), a case of ganglioneuroma of the mediastinum. Exhaustive reviews of the literature have been made by Dunn,<sup>3</sup> Blacklock,<sup>4</sup> Reid,<sup>5</sup> McFarland,<sup>6</sup> Lewis and Geschickter,<sup>2</sup> Raska and Skorpil (Fingerland<sup>7</sup>) and Wahl.<sup>1</sup> The majority of these new growths spring from the adrenal medulla, but they have been reported as arising from many parts of the body.

The three general types of sympathetic nerve tumors—ganglioneuroma, neuroblastoma and paraganglioma (pheochromocytoma)—represent cell types of different degrees or phases of differentiation from the primordial sympathetic cells, the sympathogonia. Arrested cells in any stage of development may give rise to neoplastic growth anywhere in the sympathetic system, producing a tumor of that cell type or of a mixture with numerous stages of differentiation present. Further, as has been predicted by Lehman,<sup>8</sup> Blacklock,<sup>4</sup> Lewis and Geschickter<sup>2</sup> and Wahl,<sup>1</sup> a mixture of undifferentiated cells, well developed ganglion cells, pheochromoblasts and pheochromocytes, and glial cells should be found in some cases.

Wahl and Craig<sup>9</sup> reported a case with three distinct tumors all in different stages of development, neuroblastoma, ganglioneuroma and

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From the Department of Pathology of the University of Kansas School of Medicine.

1. Wahl, H. R.: *J. M. Research* **30**:205, 1914.
2. Lewis, D., and Geschickter, C.: *Arch. Surg.* **28**:16, 1934.
3. Dunn, J. S.: *J. Path. & Bact.* **19**:456, 1915.
4. Blacklock, J. W. S.: *J. Path. & Bact.* **39**:27, 1934.
5. Reid, M.: *Ann. Surg.* **88**:516, 1928.
6. McFarland, J.: *Arch. Path.* **11**:118, 1931.
7. Fingerland, A.: *J. Path. & Bact.* **67**:631, 1938.
8. Lehman, E. P.: *J. M. Research* **31**:309, 1917.
9. Wahl, H. R., and Craig, P. E.: *Am. J. Path.* **14**:797, 1938.

ganglioneuroblastoma. Cushing and Wolbach<sup>10</sup> removed a portion of a tumor in the back of an infant and found it to be sympathicoblastoma. Laminectomy done at the same site ten years later, the child having received only a mixture of erysipelas and prodigiosus cultures (Coley's toxin) in the interim, revealed an intraspinal extradural ganglioneuroma, which was removed. A transformation had taken place by further differentiation from sympathoblasts to ganglion cells. Although various mixtures have been found, the paragangliomatous (pheochromoblastomatous) elements tend to be more in the pure state. Lewis and Geschickter,<sup>2</sup> however, reported 2 cases of malignant paraganglioma in which neuroblasts were present.

In 5 of the 51 cases of ganglioneuroma reviewed by Dunn,<sup>8</sup> in 1915, the thorax was the site. The first case of thoracic ganglioneuroma was reported by Loretz in 1870. Bigler and Hoyne<sup>11</sup> reported a case in 1934 and reviewed the previous 11 cases reported out of a total of 164 cases of ganglioneuroma. Raska and Skorpil (Fingerland<sup>7</sup>) in 1936 found 25 cases of ganglioneuroma of thoracic origin. Two proved cases have been reported since that time, bringing the total to 27. Neuroblastoma of the mediastinum is less frequent, but 13 cases have been reported out of approximately 200 cases of neuroblastoma in the entire literature. Although paraganglioma in general, including the carcinoid tumors, is the most frequent of the three types (Reid<sup>5</sup>), adult chromaffin tumors are rare, according to Philips,<sup>12</sup> who reported one of thoracic origin and made the most recent survey of the literature, to October 1940, finding only 82 cases, in 11 of which the tumor occurred outside the adrenal glands; in 9 of these it was found in Zuckerkandl's organ, which lies at the aortic bifurcation, and in only 1, in the mediastinum.

#### REPORT OF A CASE

A white boy aged 4 years was first admitted to the Children's Pavilion of the University of Kansas Hospitals (service of Dr. F. C. Neff), March 25, 1940, with swelling in the right side of the neck, noted first in December 1939. Fever, anorexia, loss of weight, occasional pain in the knees and elbow and slight cough with expectoration had been present for about six weeks. The patient was a thin, poorly nourished boy with a few enlarged, firm, knotty, slightly tender lymph nodes in the right supraclavicular fossa, exaggerated breath sounds anteriorly and an edge of the liver 2 cm. below the costal margin. There was slight secondary anemia; the red blood cell count was 3,870,000, the hemoglobin content 10.8 Gm. (70 per cent) and the white cell count 9,900 with 51 per cent polymorphonuclears. A roentgenogram of the chest revealed a mass 2 cm. in diameter in the right peritracheal area at the level of the first rib anteriorly with a larger

10. Cushing, H., and Wolbach, S. B.: *Am. J. Path.* **3**:203, 1927.

11. Bigler, J. A., and Hoyne, A.: *Am. J. Dis. Child.* **43**:1552, 1932.

12. Philips, B.: *Am. J. Path.* **30**:916, 1940.

surrounding shadow in the whole upper lung field. Biopsy of the cervical nodes was reported as showing "neuroblastoma, metastatic in lymph nodes."

On the second admission, March 30, the patient received a course of treatment with high voltage roentgen rays, directed to the right upper side of the chest and the lumbar portion of the spinal column and was discharged in four weeks showing little change, though somewhat weaker. However, the thoracic mass was considerably reduced in size.

Three months later the child began to complain of pains in the fingers, legs and toes and of soreness of the scalp. He was much weaker; large firm fixed nodules were palpable over the skull, multiple shotty nodes were present in the neck and the right supraclavicular mass was considerably enlarged. The anemia had progressed. Roentgenograms revealed extensive metastases in the skull, the ribs, the pelvis and nearly all of the long bones, with a peculiar periosteal elevation. The patient was given high voltage roentgen radiation, and anodynes were administered to control pain. Death occurred five weeks later.

*Autopsy.*—The essential findings were in the thorax. The heart was flabby and moderately dilated. The pleural cavities contained straw-colored fluid, 300 cc. on the right and 100 cc. on the left. The left lung presented no abnormality except three small round nodules, 2 mm. in diameter, in the lingula of the lower lobe; these were grayish white on section and quite firm in consistency. On the right side, occupying the upper third of the thoracic cavity, a large tumor mass, 6 cm. in diameter, compressed the upper lobe of the right lung downward and forward so that only a compact rim of this lobe remained. The middle lobe was bluish gray and definitely atelectatic, but the lower lobe showed nothing unusual. The large tumor mass was sharply demarcated from the lung, showing partial encapsulation, but it was tightly adherent to the upper three ribs on the right posteriorly and to the large firm nodular mass of lymph nodes in the supraclavicular fossa. It pressed against the upper three thoracic vertebrae medially but had not directly invaded them. The trachea was pushed slightly to the left and the innominate vein and artery were compressed and drawn out over the surface of the tumor mass. The mass itself was egg shaped, fairly smooth in contour except where invasion had taken place in the neck, pale pink and moderately firm in consistency. Sections through the middle of the tumor presented an uneven, cellular-appearing pale pink surface, while sections from the lower portion showed a patchy pale red and yellow color and an irregular patchy friable consistency alternating with soft tissue and other areas of firm dense white tissue.

The liver was enlarged, weighing 680 Gm., and multiple small grayish nodules 1 to 3 mm. in diameter were seen just beneath the capsule and on the cut surface. The thymus gland and the pancreas each contained a firm white rounded nodule.

The ribs, the vertebrae and the pelvis showed foci of diffuse thickening, dark red, rather soft in consistency, the knife cutting through them with ease as though little calcium was left. The upper ribs and the alae iliorum showed the greatest change. The periosteum was diffusely elevated, no nodules being present. Sections presented an even dark red cellular surface with few bony spicules.

*Microscopic Description.*—Sections of the lungs revealed only patchy atelectasis and healed tubercles. The hepatic parenchyma had undergone extensive fatty change, and in one section it was replaced by a densely cellular mass made up of small round or oval cells with scanty cytoplasm and dense hyperchromatic nuclei embedded in a fine fibrillar matrix. In the spleen the malpighian corpuscles were indistinct and the pulp was prominent with swelling of the sinusoidal endothelium. The pancreatic acini were shrunken and degenerated, and in one section the



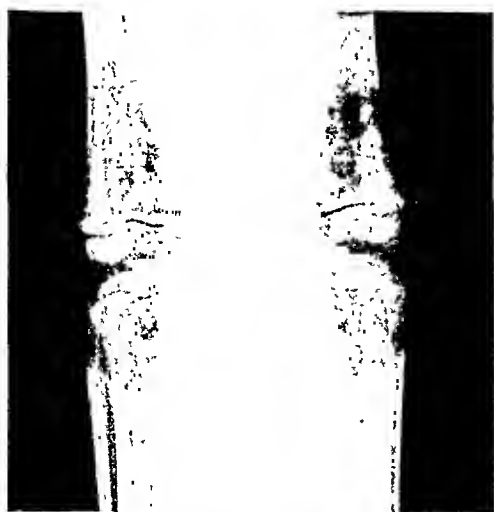


Fig. 1

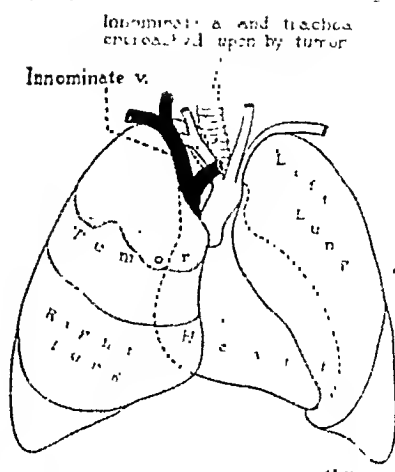


Fig. 2



Fig. 3



Fig. 4a



Fig. 4b



Fig. 5

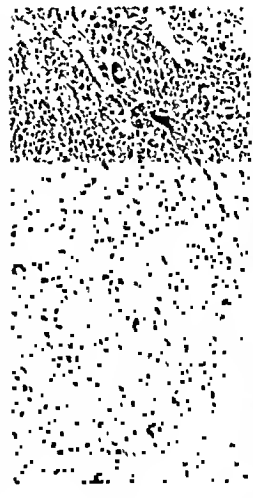


Fig. 6a

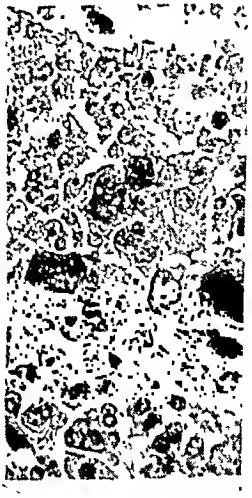


Fig. 6b

Figures 1 to 6 b  
(See legend on opposite page)

parenchyma was replaced by a partially encapsulated mass of cells quite similar to those described in the liver. Sections taken through the enlarged cervical nodes showed the same masses of cells as described in the liver.

Sections through the mediastinal tumor mass presented quite a variable picture. One section showed along one side a number of rather coarse bundles of nerve filaments. This area in which definite nerve trunks could be recognized merged gradually into an adjacent zone made up of irregular nests and clumps of small round or oval cells, all of which had dense hyperchromatic nuclei and many of which possessed delicate filamentous processes forming an irregular feltwork that tended to divide the cells into irregular nests of variable size and shape. In this same section there was another area in which a mantle of well defined neoplastic cells surrounded the blood vessels, while farther away from the vessels more degenerating tumor cells occurred. In such sections these areas of degeneration and necrotic tissue showed considerable calcification. In other sections there were considerable bundles of fine filaments, having much the appearance of neurofibrils. These could be traced for a considerable distance and extended irregularly through the tissue. No definite rosettes were seen. Frequently rather broad sheets of neurofibrillar tissue contained scattered neoplastic cells similar to those described, as well as more densely packed cellular foci. A moderate number of ganglion cells were also present in this neurofibrillar tissue.

Other areas in the tumor showed a different picture. One portion of the tissue was made up of small round or oval and stellate neoplastic-appearing cells, similar to those described in the foregoing paragraph, interspersed with broader and narrower bands of fibrillar structure showing irregular areas of hemorrhage and necrosis. In other places, however, the cells possessed a relatively abundant, finely granular cytoplasm and were elongated, fusiform or irregularly polyhedral. Considerable numbers of multinucleated giant cells were present, suggesting the structure of chromaffinoma. All transitions between this appearance and the more

#### EXPLANATION OF FIGURES 1 TO 6b

Fig. 1.—Roentgenogram of the bones of both knees showing the metastases in the femurs and the tibias, especially near the knee joints.

Fig. 2.—A diagrammatic sketch of the heart and lungs showing the location and relations of the tumor.

Fig. 3.—Low power photomicrograph ( $\times 61$ ) showing clumps and clusters of sympathogonia invading a feltwork of neurofibrils containing degenerated ganglion cells. This was taken from the main tumor mass in the upper left side of the thorax.

Fig. 4a.—Lower power photomicrograph showing bundles of neurofibrils, taken from the main tumor mass.  $\times 61$ .

Fig. 4b.—Lower power photomicrograph of an area in the main tumor mass showing ganglion cells embedded in a neurofibrillar stroma.  $\times 61$ .

Fig. 5.—Low power photomicrograph of another portion of the tumor representing the structure of the bulk of the tumor sheets and masses of sympathicoblasts, some of which give the reactions of pheochromocytes.  $\times 61$ .

Fig. 6a.—Low power photomicrograph of a field from the main tumor showing masses of pheochromocytes, with variation in cell shape and type, and secondary necrosis. Multinucleated giant cells are also seen.  $\times 61$ .

Fig. 6b.—High power photomicrograph from an area similar to that shown in figure 6a. Note the large multinucleated giant cells with hyperchromatic nuclei. Some of these cells show a positive reaction with silver stains.  $\times 290$ .

undifferentiated types of cells could be found. There was a tendency to separate the masses of cells into poorly defined lobules, partly separated from one another by delicate septums of connective tissue containing endothelium-lined spaces, some of which were filled with red cells. An affinity for silver salts was seen in the paragangliomatous cells (methods of Bielschowsky and of Wilder), and occasionally the cells of sections of tissue fixed in Zenker solution contained definite light yellow chromaffin granules.

Transverse sections through the ribs showed extensive replacement of the osseous tissue by neoplastic tissue of the neuroblastomatous type. No pronounced types of cells were recognized. They appeared undifferentiated and often showed a small bit of cytoplasm at one side of the nucleus or showed nuclei embedded in the fibrillar stroma. There were considerable congestion and hemorrhage with necrosis. The remnants of bone lay at the periphery of the rib.

*Final Diagnosis.*—Sympathogonioma (mixed neuroblastic and pheochromoblastic) with metastases to the skull, ribs, vertebrae, pelvis, long bones, liver, lung, pancreas, thymus, upper thoracic wall and mediastinal cervical lymph nodes; acute dilatation of the heart; fatty change of the liver; acute splenitis; chronic focal pleurisy.

This tumor was unusual chiefly in that it was made of a widely diverse assortment of cells of sympathetic origin in all stages of differentiation from sympathogonia to adult ganglion cells and adult chromaffin cells. It obviously had its origin from the neurocytes of the sympathetic trunk in the upper thoracic region. The term "neuroblastoma" includes both sympathogonioma, composed of undifferentiated sympathetic cells, and the sympathicoblastoma, containing more differentiated elements. Differentiation was shown chiefly toward paraganglionic cells rather than toward neuroblasts, but in different sections either of these two cell types might be seen to be predominant. In a few areas nonmedullated nerve fibers and definite well differentiated ganglion cells could be recognized. Transitions could be seen between the primitive nerve cells and the chromaffin cells, like those of the adrenal medulla, some fields showing characteristic giant cells seen in the primary paraganglionic tumors of the medulla. Typical rosettes were not seen, but the fine fibrils were present often in bundles. Lewis and Geschickter<sup>2</sup> found rosettes in one third of the cases they studied, and Wahl<sup>1</sup> reported them in one half of the cases described in a previous paper. The metastases that were present were all of the embryonic neuroblastomatous type, the differentiated elements being located in the main tumor mass.

The location of a tumor of this type is another point of interest, since most sympathetic tumors have been found in the adrenal gland or the celiac region.

#### COMMENT

Unless mixed with undifferentiated cellular elements, the paraganglioma and the ganglioneuroma do not metastasize. Crile and Ball<sup>13</sup>

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13. Crile, G. W., and Ball, R. P.: Surg., Gynec. & Obst. 48:449, 1929.

stated that only cells of the most embryonic type are ever cancerous and that this type becomes increasingly benign as it reaches the adult stage. This is borne out by the age incidence for each of the three tumor groups, the average age for neuroblastoma being  $2\frac{1}{2}$  years (Blacklock<sup>4</sup>; Frew<sup>14</sup>; Reid<sup>5</sup>), that for ganglioneuroma 19 years in the 52 cases reviewed by Reid,<sup>5</sup> and that for paraganglioma the fifth decade, according to Lewis and Geschickter.<sup>2</sup> As a general rule, the younger the child the greater the degree of malignancy and the shorter the duration of life. With neuroblastoma the average length of life after onset was six months in the 40 cases of Lewis and Geschickter<sup>2</sup> and eight to nine weeks in Blacklock's<sup>4</sup> cases.

In neuroblastoma, widespread secondary involvement of bones—not only the skull, the ribs and the sternum but the long bones down to the hands and feet—is of frequent occurrence, and histologically the secondary growths are quite similar to primary growths with the exception that fibrils and rosettes are not usually seen. The mode of this spread has been the subject of much discussion. Frew<sup>14</sup> suggested a direct lymphatic spread from the primary neoplasm to the skull; Cohn (Blacklock<sup>4</sup>) stated that the greater vascularity of the ribs and of the skull of the infant accounts for the bony metastases but did not explain the pathway of implantation; Lehman<sup>8</sup> expressed the belief that the cells gain access to the greater circulation via a patent foramen ovale; others have assumed that the small cells could pass through the pulmonary capillaries to be disseminated by the general blood stream. Batson<sup>15</sup> in a recent publication provided a most plausible explanation for these bony metastases by demonstrating a hitherto little known circulation through the valveless veins in a great plexus about the vertebral column. By injection experiments he demonstrated that roentgen opaque material flows easily through this plexus, even at great distances from the sites of injection, directly to most of the bones of the body. The fact that the sympathetic ganglions and their gray and white rami communicantes lie in such close relationship to the vertebral bodies and thus in intimate association with the vertebral venous plexus makes this method of bony metastasis in the cancers of sympathetic origin seem quite likely.

#### SUMMARY

Of the three general types of sympathetic nerve tumors, various mixtures may be found in a given tumor, but the paragangliomatous elements tend more to be found in a pure state.

The thorax is not a frequent site of origin of these tumors; it has been reported as the primary site in 27 cases of ganglioneuroma, 13 of neuroblastoma and 2 of paraganglioma.

14. Frew, R. S.: *Quart. J. Med.* **4**:123, 1911.

15. Batson, O. V.: *Ann. Surg.* **113**:138, 1940.

The history and autopsy findings in the case of a 4 year old boy with a unique thoracic tumor bearing a mixture of all neurogenous elements with a preponderance of pheochrome cells are given. This tumor is of particular interest, as it not only showed undifferentiated neurocytes but illustrated their differentiation into ganglion cells, nerve fibrils and pheochromocytes, with an unusual accumulation of the latter; in fact, this tumor is unique in that it showed such a predominance of pheochromoblasts with the undifferentiated tissues, a finding not heretofore described in the thorax. It also showed cancerous parts, metastasizing widely and comprised of sympathogonia, and parts composed of both neoplastic differentiated nerve elements and pheochromocytes. Metastasis to bones by way of the paravertebral plexus of veins is suggested.

# EFFECT OF MECHANICAL FORCE ON THE SKELETAL LESIONS IN ACUTE SCURVY IN GUINEA PIGS

RICHARD H. FOLLIS JR., M.D.

BALTIMORE

When one is considering a diagnosis of scurvy based on histologic changes at the ends of the growing bones of an infant or an experimental animal, there are certain criteria on which one leans heavily. Fractures of the "lattice," together with the presence of the *Trimmerfeldzone* (zone of detritus) and the *Gerüstmark* (framework marrow), make the presence of ascorbic acid deficiency a virtual certainty. Many who examine the bones of children at autopsies are probably loath to make a diagnosis of scurvy in the absence of these classic criteria. The fundamental disturbance in scurvy, however, is a failure of normal fibroblastic, osteoblastic and odontoblastic activity so that collagen, osteoid and dentin are not formed.<sup>1</sup> The relationship of this primary pathologic change to the final histologic picture is therefore not entirely clear. It has seemed fairly evident, however, that in scurvy, and in rickets as well, the mechanical factors of stress and strain must exert great influence on the final histologic picture. Park, Guild, Jackson and Bond<sup>2</sup> have commented on the importance of the effect of strain on the histologic changes encountered in the bones of scorbutic children. It appears, then, that if these mechanical effects could be prevented, the ensuing lesions in the bones might be modified. The present report deals with such an experimental study.

## MATERIALS AND METHODS

Guinea pigs weighing 150 to 300 Gm. were fed a stock diet supplemented with carrots and ascorbic acid for a week or more. All sources of vitamin C were then excluded. A few days later a narrow plaster bandage was wound about one of the hindlegs. Beginning at the ankle, the winding was continued up to the thigh and then on up about the abdomen. This cast immobilized the leg almost completely, while the opposite one was allowed freedom of motion. The animals were killed at intervals of from ten to twenty days. The lower ends

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From the Department of Pathology of the Johns Hopkins University School of Medicine.

1. (a) Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926. (b) Wolbach, S. B.: Am. J. Path. **9**:689, 1933.

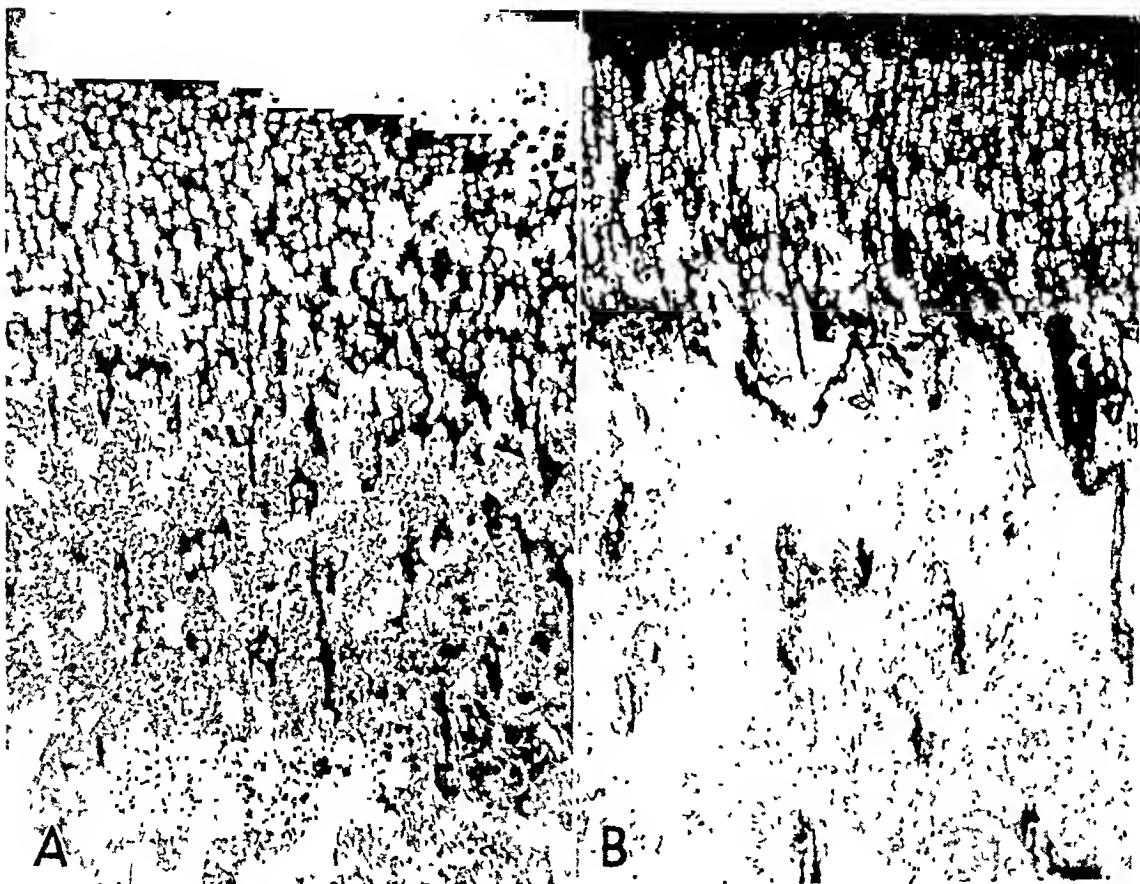
2. Park, E. A.; Guild, H. G.; Jackson, D., and Bond, M.: Arch. Dis. Childhood **10**:265, 1935.

of the femurs and the upper ends of the tibias were decalcified, embedded in paraffin or celloidin (a concentrated preparation of pyroxylin) and stained with hematoxylin and eosin.

#### EXPERIMENTAL RESULTS

The following description is a composite picture of the findings in a number of animals. The figure illustrates quite clearly the difference in the two extremities of each animal.

At the cartilage-shaft junction of the immobilized leg the cartilage appeared normal (*A* in figure). Beneath the zone of proliferative



*A*, cartilage-shaft junction of an immobilized leg of a guinea pig. Note the abnormally broad "lattice," which has no bone on it. Note also the marrow cells up to and between the columns of the "lattice." *B*, cartilage-shaft junction of the opposite leg of the same animal. Note the fractures of the "lattice" and the absence of marrow cells beneath it. Here there are connective tissue and red blood cells.

cartilage cells there was a broad "lattice" of calcified cartilaginous matrix, which was wider than one encounters normally. This matrix was arranged longitudinally and horizontally; the spaces thus formed indicated where cartilage cells had been destroyed. Blood vessels were found between the interstices of the matrix, and there were the usual numbers of connective tissue cells in such locations as well. It was

evident that invasion of the normal-appearing cartilage cell columns was taking place as usual. The abnormal feature, however, was that the "lattice" was not being destroyed. No osteoid or bone was found on the "lattice" until one reached its base, i. e., the place where the shaft began. Here there was a normal transition between the "lattice" and the bone. The absence of osteoid deposit on those portions of the "lattice" where osteoid should have been was the second pathologic feature. In the marrow spaces there was extensive erythropoietic and myeloid activity; the marrow cells came all the way up to the junction of "lattice" and shaft. No fractures of the "lattice" were observed except at the corners in a few animals. It was thought that these were due to incomplete immobilization of the knee joint in the cast. There was no proliferation of connective tissue cells nor was there any pink-staining "fibrin-like" material or hemorrhage.

In the animals whose legs had been allowed motion some rather marked differences were found (*B* in figure). The epiphysial cartilage was similar to that of the opposite leg. The "lattice" of calcified matrix in some animals was just as wide; in others it was narrower. This depended on the number and the severity of the fractures that were found. The points of fracture were characterized by an accumulation of pink-staining "fibrin-like" material, together with numerous osteoblastic cells, about the spicules of fractured "lattice." Giant cells were encountered about some of the broken remnants. There were hemorrhages as well. Marrow cells were not found at the cartilage-shaft junction, but there was a wide zone in which only connective tissue cells remained. Thus there emerged the classic picture of scurvy with fractures, *Trümmerfeldzone* and *Gerüstmark*.

#### COMMENT

It seems clear that when one eliminates the usual stresses and strains that accompany motion of the extremities, the histologic picture of the skeleton in ascorbic acid deficiency is greatly altered. Such a study indicates that all the usual criteria which one calls on in establishing a diagnosis of scurvy are entirely secondary to the initial cessation of osteoblastic activity as indicated by a failure to destroy the calcified cartilaginous matrix and to form osteoid.

There are several points that might be stressed. The pink-staining "fibrin-like" material that appears about fractures has been interpreted by students of scurvy in various ways. Aschoff and Koch<sup>3</sup> thought it was fibrin, while Höjer<sup>4</sup> decided it was bone of an inferior type. Wol-

3. Aschoff, L., and Koch, W.: *Skorbut, Eine pathologisch-anatomische Studie*, Jena, Gustav Fischer, 1919.

4. Höjer, J. A.: *Acta pædiat. (supp.)* 3:8, 1924.



bach and Howe<sup>1a</sup> thought it "had as its basis a product of the cells of the *Gerüstmark*, probably liquid until added to by other materials from the blood plasma or cartilage matrix resorption." The failure to observe this material in the immobilized bones would serve to indicate that its appearance is secondary to the fractures and possible rupture of capillaries and that it does not represent a defective product elaborated by the osteoblasts as Wolbach and Howe<sup>1a</sup> suggested, unless these cells are stimulated to an attempt at healing.

In this study of acute conditions no disturbance in the invasion of the cartilage cell columns by capillaries was observed. Wolbach's<sup>1b</sup> experiments would indicate that in the scorbutic state there is a failure of endothelial cells to form capillaries just as there is a failure of connective tissue cells to form intercellular substances. Experiments similar to those reported here but of a more chronic nature might bring out this defect.

What the stimulus for the disappearance of the myeloid elements and the appearance of the *Gerüstmark* may be is difficult to decide. The occurrence of the fractures with the subsequent hemorrhage and connective tissue proliferation coincides with the disappearance of these cells from this area. Changes in the environment, such as anoxemia or differences in  $p_{H}$ , may be important.

These experiments seem to indicate that one might be able to diagnose scurvy in children and animals on the basis of a persistent "lattice" and lack of bone formation. My associates and I will discuss this question in another place and can say now only that a positive diagnosis on such a basis is in our experience well nigh an impossibility.

It should also be pointed out that a persistent "lattice" with little or no bone deposited on it is not specific for ascorbic acid deficiency. In congenital syphilis similar changes are encountered.<sup>5</sup>

#### SUMMARY

A study has been made of the effect of immobilizing an extremity on the ensuing histologic picture of experimental scurvy in guinea pigs. It was found that the classic picture, with fractures, *Trimmerfeldzone* and *Gerüstmark*, failed to appear. This indicates that these are secondary to the failure of bone to be deposited on the delicate calcified cartilaginous matrix.

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5. Park, E. A.; Jackson, D.; Goodwin, T. C., and Kajdi, L.: *J. Pediat.* **3**:265, 1933.

# HISTOLOGIC OBSERVATIONS ON THE CHANGES IN THE BRAIN IN ROCKY MOUNTAIN SPOTTED FEVER

I. MARK SCHEINKER, M.D.

CINCINNATI

A histologic study of a victim of Rocky Mountain spotted fever is presented in which particular attention was paid to a special pericapillary cellular reaction.

A 67 year old white woman was admitted to the Cincinnati General Hospital Jan. 24, 1941. About three weeks previously she and her two grandchildren visited in Kentucky. Two weeks later she became ill with nausea, vomiting and diarrhea. About the same time the two grandchildren became ill. A few days later the patient, as well as the children, presented a rash which appeared on the ankles and then spread rapidly over the rest of the body. For the next five days the patient was delirious and had a high fever.

On examination the patient had a generalized macular hemorrhagic eruption over the entire body except the face. The blood pressure was 94 systolic and 60 diastolic. Respiration was labored and rapid, and the heart sounds were rapid and feeble. The temperature was 103 F. and the patient was delirious. She was hypersensitive to touch. There was rigidity of the neck, with hyperactive tendon reflexes and bilateral extensor plantar responses. The temperature rose steadily and finally reached 106.8 F. The patient died after twenty hours in the hospital. The red blood cell count was 6,160,000; the white cell count, 10,000, with 82 per cent polymorphonuclear leukocytes. The initial reading of the cerebrospinal fluid pressure was 110 mm. of water. The fluid contained 11 lymphocytes and 21 red blood cells per cubic millimeter. The result of the Pandy test was normal. The Wassermann reaction of the blood was negative, and the blood cultures remained sterile. The Weil-Felix reaction in the blood was positive.

The gross pathologic abnormalities were diffuse pulmonary edema and congestion, congestion of the liver, the spleen, the kidneys, the fundus of the stomach, the small and the large bowel and the meninges, and petechial hemorrhages of the skin, the liver, the kidneys and the mucosa of the cecum.

The following areas of the brain were removed for microscopic examination: the right basal ganglions with cortex from the sylvian fossa and the hippocampal gyri, several other areas of the cortex and portions of the pons and the medulla.

The histologic examination revealed miliary granulomas and capillary changes. These lesions varied in number and in degree in different regions of the brain.

The most striking manifestation of the pathologic process was the presence of small focal lesions in the form of miliary granulomas widely scattered throughout the brain substance. The granulomas as seen in cross section were generally circular or ovoid and were composed of compact accumulations of large irregular

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From the University of Cincinnati College of Medicine and the Laboratory of Neuropathology of the Cincinnati General Hospital.

polygonal or oblong cells (fig. 1 *A*). The cells usually varied considerably in size and shape. The cytoplasm stained lightly and homogeneously except occasionally, when it appeared coarsely granular. The cell nuclei were round, oval or oblong and usually poor in chromatin. The central part of some of the

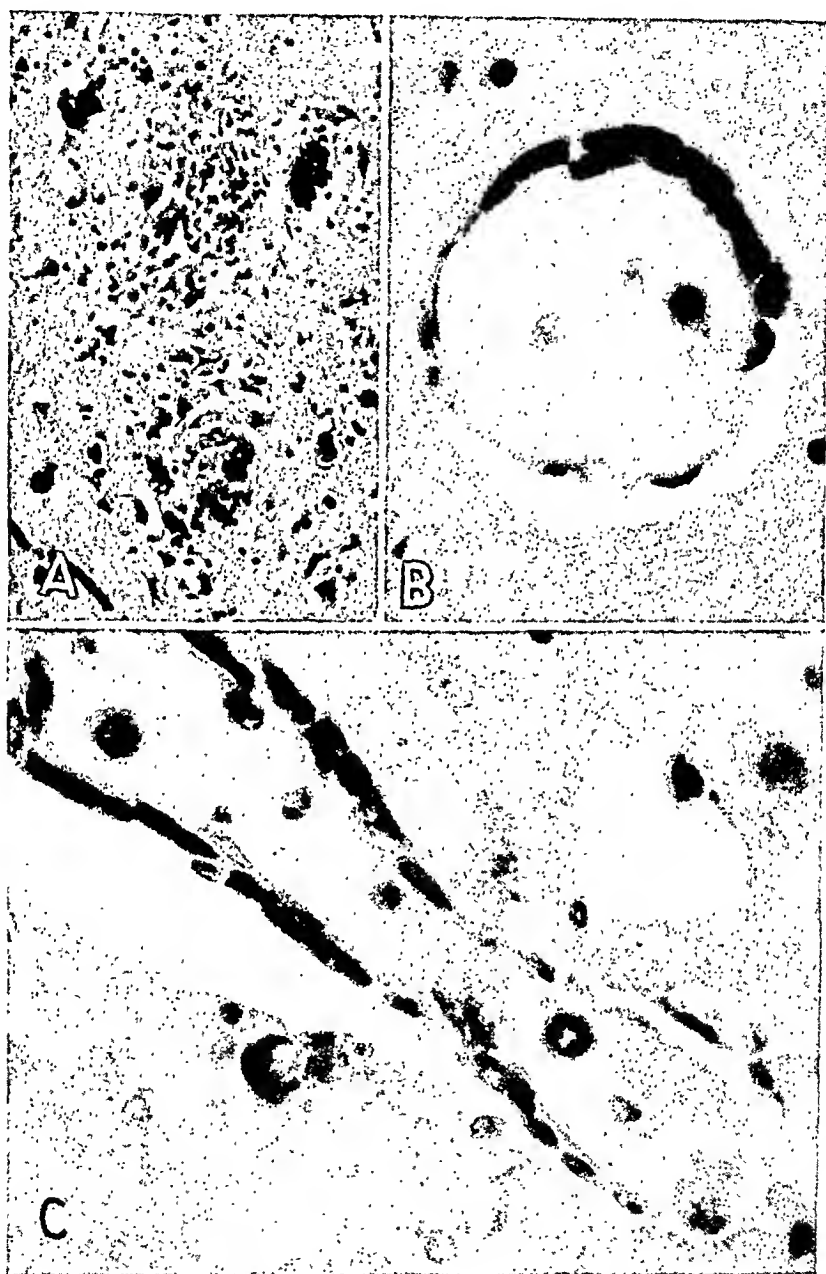


Fig. 1.—*A*, miliary granuloma formed by compact accumulation of irregular polygonal and oblong cells. Cresyl violet;  $\times 135$ . *B*, pericapillary proliferation of a small number of large mononuclear cells. Note the normal appearance of the endothelial cells. Cresyl violet;  $\times 165$ . *C*, pericapillary cell proliferation. Cresyl violet;  $\times 165$ .

granulomas appeared edematous, and the cells were degenerated so that their form and structure were not recognizable. Inflammatory phenomena and hemorrhages were not found in the granulomas or in the surrounding brain tissue.

The majority of the granulomas contained capillaries. Only a few were avascular. It appeared probable that an associated vessel might have been found in each granuloma if serial sections had been made. The endothelial layer of the capillary was normal except for a moderate degree of hyperplasia. There was marked cell proliferation in the adventitia, which was infiltrated by large mononuclear cell elements that in their structure resembled those of the neighboring granuloma cells. There seemed to be transitions from the cells derived from the capillary wall to the granuloma cells.

The Cajal gold sublimate stain revealed only an occasional astrocyte among the other cells of the granuloma. This cell probably belonged to the preexisting glia. There appeared to be no relation between it and the granuloma cells. About the margins of some of the granulomas there was a slight accumulation of hyperplastic microglia cells. There was no evidence of transition from these cells to the granuloma elements.

Where granulomas were present, nerve cells, myelin sheaths and nerve fibrils had practically disappeared. In the tissue surrounding the granulomas the nerve cells were fairly well preserved.

The extensive and characteristic lesions of the capillaries of the brain deserve attention. The earliest change consisted of a pericapillary proliferation of a small number of large mononuclear polygonal cells around a limited sector of a capillary (fig. 1 *A* and *B*). These cells, slightly hypertrophied, contained vesicular nuclei and resembled the so-called Rouget cells lining the capillary wall. More extensive and presumably more advanced lesions affected the entire circumference of the capillary, so that there was concentric thickening of the walls of the vessel due to an accumulation of large numbers of mononuclear cells (fig 2 *B* and *C*). No lymphocytes or plasma cells were present. The endothelial cells which line the capillary lumens were not recognizable in this later stage.

In the longitudinal sections of some capillaries it was noted that the mononuclear cells originated from the adventitial capillary tunic and infiltrated the surrounding nerve tissue, forming a small granuloma (fig. 2 *A*). The majority of the diffusely scattered smaller aggregations of cells, which evidently represented the early stage of granuloma formation, were formed around capillaries. The proliferation of the capillary wall could be regarded as their origin (fig. 2 *A*). Many of the granulomas revealed early signs of dissolution and necrosis, which destroyed the cells and the capillary wall from which they originated (fig. 2 *B*). In the more advanced lesions in which the wall of the blood vessel as well as the granuloma cells were degenerated, the structure of the granuloma and its connection with the capillary could not be recognized. No noteworthy changes were present in the arteries or in the veins of the brain. The majority of the smaller veins and capillaries revealed signs of dilation with passive hyperemia, and occasionally they were surrounded by small perivascular hemorrhages.

In scarlet red preparations the ganglion cells throughout the brain cortex exhibited slight signs of fatty degeneration. Granules of fat were present in the adventitia of the small arteries. No trace of inflammation was found in the meninges, the cortex or the white matter. No areas of primary demyelination were found, and only here and there was there some rarefaction of the myelin network in the main pathways. In some areas in which the granulomatous formation was more marked, both progressive and regressive glial changes were present. The degenerative changes consisted in swelling of the cell body and tumefaction of the processes, which were undergoing gradual disintegration. The

progressive changes were a slight increase in the number of microgliocytes, especially pronounced in the vicinity of the granuloma. No special search for microorganisms (rickettsias) was made, though minute basophilic inclusions were occasionally found in the media and the adventitia of some arterics.

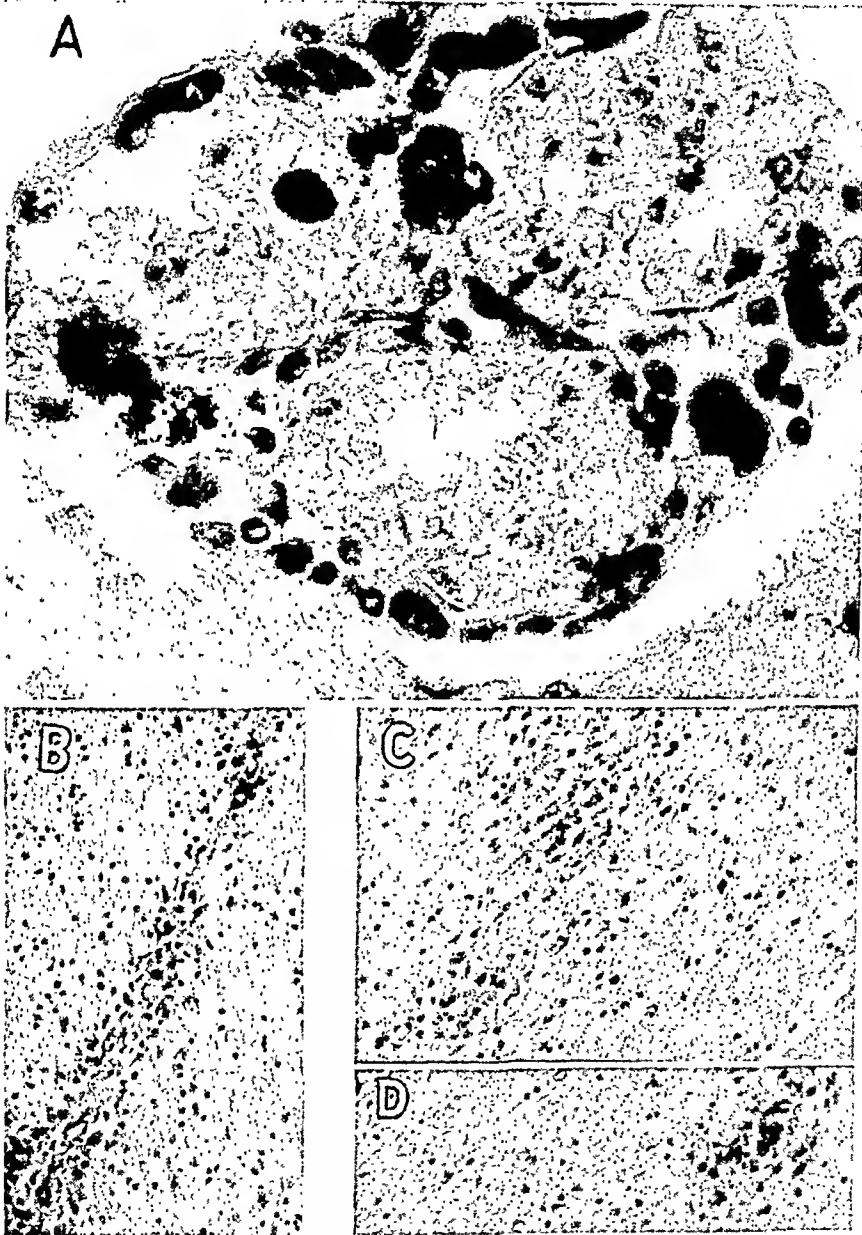


Fig. 2.—*A*, advanced cell proliferation around the capillaries with concentric thickening of the wall. Cresyl violet;  $\times 135$ . *B*, longitudinal section of a capillary which is surrounded by a large mass of mononuclear cells from the adventitial tunic of the blood vessel. These cells infiltrate the surrounding nerve tissue and give rise to granuloma. Cresyl violet;  $\times 135$ . *C*, and *D*, small perivascular granulomas with early signs of dissolution and necrosis of the granuloma cells. Cresyl violet;  $\times 135$ .

In summary it appears that the most constant and uniform lesion in the brain was the widely disseminated granuloma formation. In the majority of instances the granuloma was in direct contact with a capillary, from the wall of which its cells appeared to be derived. In some areas, however, the cellular agglomeration appeared avascular and showed no obvious relationship to a blood vessel. Probably in these areas necrosis of the vessel walls had occurred.

The cerebral histologic changes of Rocky Mountain spotted fever in man have been reported only casually. Most descriptions have dealt with changes observed in the central nervous system as observed in animals. In the majority of 20 cases of Rocky Mountain spotted fever commented on by Wolbach<sup>1</sup> the central nervous system had been reported as normal. Wolbach's study demonstrated the remarkable specificity of the micro-organism (*rickettsia*) of Rocky Mountain spotted fever for the peripheral circulation. Wolbach recorded fully the lesions in the various organs (except the central nervous system) in man and experimental animals. He noted that "the vascular lesions are at first essentially proliferative (endothelium) followed by necrosis of small groups of cells." The character and evolution of the rash with the cutaneous sequelae (necrosis or gangrene) were explained by the lesions of the blood vessels.

Similar observations were earlier recorded by Le Count,<sup>2</sup> in the skin, the liver, the kidney, the spleen and the adrenal glands. Lillie<sup>3</sup> described the findings in the central nervous system as determined in a study of 5 autopsy records. Three different types of brain lesions were recorded: "Those involving vessels and their sheaths, focal proliferative lesions in the brain substance and focal necroses." Similar vascular and focal lesions were present in cases reported by Pinkerton and Maxcy<sup>4</sup> and Harris.<sup>5</sup> Changes of the same kind as those seen in the brain of man have been produced in animals infected with Rocky Mountain spotted fever (Lillie, Dyer and Topping<sup>6</sup>). Hassin<sup>7</sup> described mild inflammatory and degenerative changes in the brain in a fatal case of Rocky Mountain spotted fever. The histologic findings were classified by him as nonsuppurative meningoencephalitis and designated as being "not typical of any particular form of encephalitis."

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1. Wolbach, S. B.: *J. M. Research* **41**:1, 1919.

2. Le Count, E. R.: *J. Infect. Dis.* **8**:421, 1911.

3. Lillie, R. D.: *Pub. Health Rep.* **46**:2840, 1931.

4. Pinkerton, H., and Maxcy, K. F.: *Am. J. Path.* **7**:95, 1931.

5. Harris, P. N.: *Am. J. Path.* **9**:91, 1933.

6. Lillie, R. D.; Dyer, R. E., and Topping, N. H.: *Pub. Health Rep.* **54**: 2137, 1939.

7. Hassin, G. B.: *Arch. Neurol. & Psychiat.* **44**:1290, 1940.

## COMMENT

It seems proper to attempt to correlate the cerebral findings in this case with those described in the literature. The formation of the miliary granulomas seems to be a constant and characteristic finding in cases of Rocky Mountain spotted fever. Lillie<sup>3</sup> described the granulomas as accumulations of "leptochromic glia nuclei in single rows along the sheaths of vessels." Hassin<sup>7</sup> noted that the cells of the granulomas were "not fibroblasts or microgliocytes but probably oligodendrocytes or glia nuclei." The same type of lesion has been repeatedly reported in cases of typhus fever; it has been observed in cases of epidemic encephalitis, toxoplasmic encephalitis and Borna disease and in cases of encephalitis associated with trichinosis and that associated with malignant endocarditis. In some forms of encephalitis (*Trichina* and *Toxoplasma*) the miliary granulomas contain parasites. These lesions are probably a manifestation of a local tissue reaction to the presence of an infectious invader.<sup>8</sup>

There are several bits of evidence that indicate the miliary granulomas are not glial in origin: their development around blood vessels, the constant presence in them of large mononuclear cell elements which are not stainable with specific methods for staining glia and the resemblance of these cells to the large mononuclear cells found in the circumference of the capillary and presumably derived from the adventitial tunic of the vessel wall. It was possible to make a clearer interpretation than heretofore of the nature of the pericapillary cellular proliferation in the case under discussion because of the absence of perivascular inflammatory reaction.

It seems proper to conclude that the granuloma cells are mesodermal in origin and that probably they are derived from the adventitial tunic of the capillary.

In support of the mesodermal origin of such cells is the work of Wolf and Cowen,<sup>9</sup> who described miliary granulomas in toxoplasmic encephalitis as follows: "In most cases the granulomas surrounded or were in contact with a small blood vessel from the wall of which its epitheloid cells appeared to be derived." Harris found that "in addition to the true perivascular cellular infiltration many of the arteries within the brain tissue show an accumulation of monocytes in the stroma which accompanies them in their course." Wolbach<sup>1</sup> mentioned that "the earliest lesion in the vessels is a collection of large mononuclear phagocytic cells (endothelial cells) over an area of swollen and degenerated endothelium of the intima." On the other hand, in cases

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8. Hassin, G. B., and Diamond, F. B.: *Arch. Neurol. & Psychiat.* **15**:34, 1926. Hassin.<sup>7</sup>

9. Wolf, A., and Cowen, D.: *Bull. Neurol. Inst. New York* **7**:266, 1938.

of granuloma formation in typhus fever the origin of the granulomas has been ascribed to necrosis of the endothelium of the blood vessels, which was thought to be the primary phenomenon (Jahrisch<sup>10</sup>; Herzog<sup>11</sup>; Bauer<sup>12</sup>; Ceelen<sup>13</sup>; Gross<sup>14</sup>).

#### SUMMARY

The histologic observations in a fatal case of Rocky Mountain spotted fever are reported. The changes consisted of a proliferative cellular reaction of the capillary wall and formation of scattered miliary granulomas. The granulomas and the proliferative reaction, made up of large mononuclear cells, appeared to be derived from the capillary adventitia and therefore to be of mesodermal origin. The degenerative phenomena consisted of lesions of the ganglion cells combined with a mild focal microglial proliferation in the neighborhood of the granulomas.

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10. Jahrisch, A.: *Deutsches Arch. f. klin. Med.* **126**:270, 1918.
  11. Herzog, G.: *Centralbl. f. allg. Path. u. path. Anat.* **29**:97, 1918.
  12. Bauer, E.: *München. med. Wchnschr.* **63**:541 and 1243, 1916.
  13. Ceelen, W.: *Ergebn. d. allg. Path. u. path. Anat.* **19**:313, 1919.
  14. Gross, W.: *Virchows Arch. f. path. Anat.* **242**:452, 1923.



# Case Reports

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## SEMINOMA DEVELOPING IN AN UNDEVELOPED GENITAL ANLAGE

H. GIDEON WELLS, M.D., CHICAGO

Twenty-five years ago I performed a necropsy in a case the like of which I had not seen or heard of previously. Since then I have waited in vain to see a report of a similar case, but none has appeared. Therefore it seems to be about time to put the case on record.

### REPORT OF A CASE

A 47 year old press feeder, came to the service of Dr. Wilber E. Post at the Cook County Hospital, Chicago, on Feb. 13, 1917 and died on March 10, 1917. He complained of loss of weight and weakness which had prevented him from doing any work for five months. He had a pain in the left side in the posterior axillary line at the level of the lowest rib. He did not cough, there was no edema, he had never been married and denied that he had ever had a venereal disease. In his reactions and behavior he was decidedly infantile. Hypospadias was present, there was no right testicle in the scrotum and the left testicle was decidedly atrophic. The lower border of the liver was 3 fingerbreadths below the costal margin and overlay a large retroperitoneal mass. There was an increased prominence of the hypogastric and epigastric veins. Death occurred from terminal hypostatic bronchopneumonia.

Necropsy revealed the following significant findings: The body was that of a very small (5 feet [152.5 cm.] tall), poorly nourished and poorly developed man, looking somewhat younger than the stated age. The beard, mustache, axillary and pubic hair were very sparse. The face appeared childish. The superficial lymph nodes were not palpable. There was noticeable exophthalmos. The right testicle was not present in the scrotum, and there was no scar to indicate an operative removal. The left testicle was present in the scrotum and about one third the normal size. The penis was only 2 cm. long, and the urethra, discharging a bloody urine, opened between the base of the penis and the scrotum; there was no urethra whatever in the penis.

When the abdominal cavity was opened, there was found no undescended testicle at any site, no right spermatic cord, no right seminal vesicle. The prostate was about half the normal size, and the right half was merely a fleshy mass. The left seminal vesicle was present in the form of a large hollow sac, and the left testicle was reduced to a small mass, 1.5 cm. in diameter, with a small epididymis.

A large tumor mass was located retroperitoneally and nearly symmetrically, although a little larger portion was on the left side. The stomach was pushed to the left and was adherent to the tumor mass, the pylorus being stretched out so that it resembled the duodenum. The tumor mass lifted up the root of the mesentery and ascended up through the root of the diaphragm to the level of the seventh dorsal vertebra, the portion in the thoracic cavity extending 10 cm. above the diaphragm and being 15 cm. in breadth. The entire mass was 35 cm. long,

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From the Department of Pathology of the University of Chicago.

25 cm. wide at its widest part and 20 cm. thick. On the anterior surface was a groove containing the portal vein, which evidently was not greatly compressed, since there was no ascites. The tumor tissue was adherent to the vertebral fascia and infiltrated the perivertebral tissues and the bodies of the vertebrae themselves. It consisted of large lobulated masses of pinkish white tissue without evident areas of necrosis, its consistency being somewhat firmer than normal liver tissue, and the weight was 2,800 Gm. The aorta and the vena cava ran through the mass but were not occluded by it.

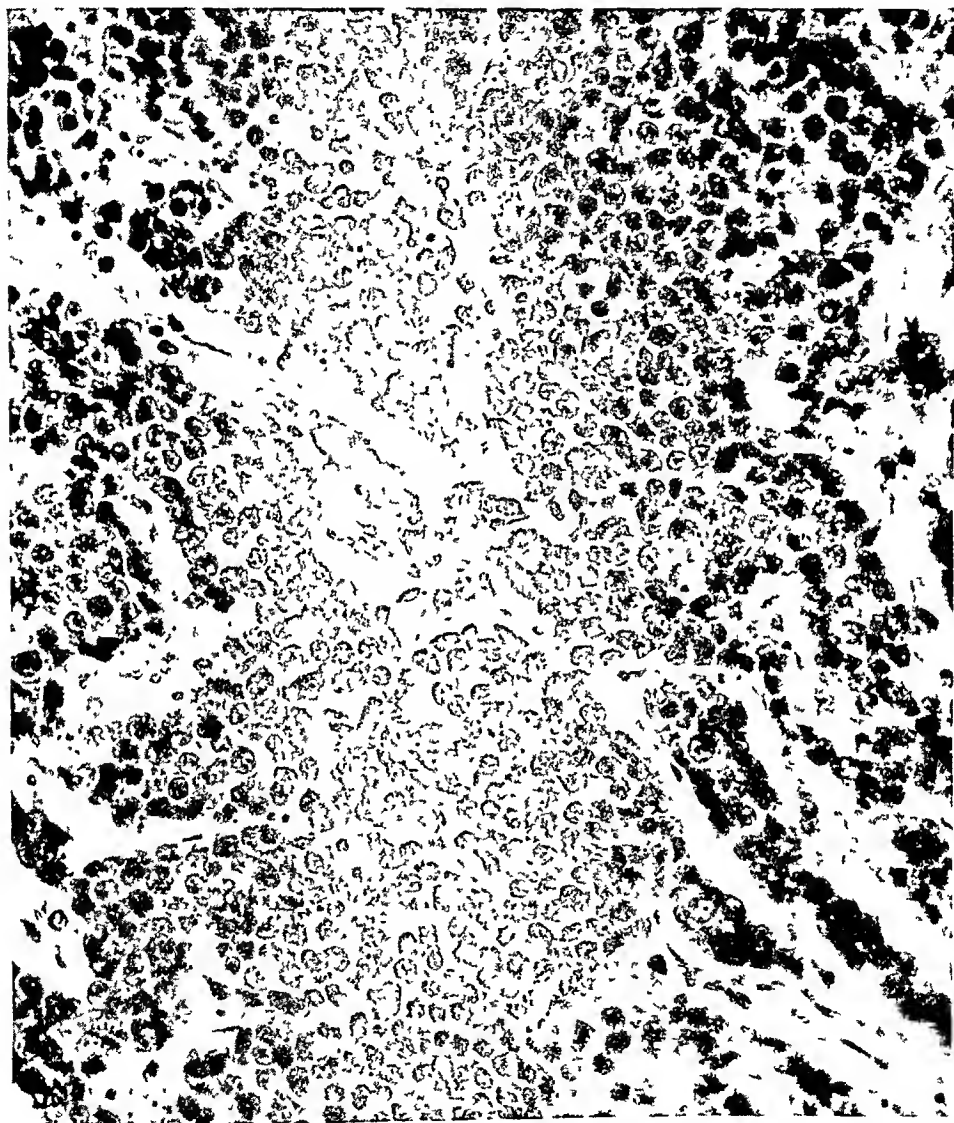


Fig. 1.—Section of the primary tumor ( $\times 235$ ) showing the seminoma-like structure.

The left kidney was not adherent or involved with the tumor in any way and appeared practically normal. The right kidney was forced downward and spread out like a capsule over a mass of tumor tissue entering the hilus but not infiltrating the kidney tissue. The urinary bladder was distended but appeared normal. The right adrenal was adherent to the tumor but not involved. The left adrenal was flattened and spread over a nodule of tumor tissue but not infiltrated. The pancreas

was spread out flat and thin over the tumor but was not infiltrated. There were no remote metastases, but three lymph nodes about the base of the bladder were slightly enlarged.

There was a general small size of the viscera (heart, 150 Gm.; liver, 800 Gm.; spleen, 80 Gm.). The gallbladder was packed with 250 gallstones. The left lung showed hypostatic bronchopneumonia.



Fig. 2.—Section of the prostate ( $\times 10$ ) showing the presence of undeveloped tubules in the left half and the absence of tubules in the right half.

Microscopically, the tumor was a large-celled alveolar growth (fig. 1), resembling typical testicle tumors, with many atypical mitotic figures—in structure a seminoma. The pelvic lymph nodes and the vertebrae adjacent to the tumor were extensively invaded by the same sort of growth. The left testicle showed a condition of extreme atrophy with hyalinization of the basement membrane of the seminiferous tubules and no germinal epithelium present at all; the interstitial

tissue contained but few cells of Leydig. The left epididymis was infantile in character. Most interesting was the prostate, for in the right half were no tubules whatever, only nonstriated muscle, while the left half had a few tubules of infantile character (fig. 2). Obviously, the entire right genital anlage had failed to develop, while the left had developed but partially. Apparently, the undeveloped genital anlage had remained quiescent for forty odd years and then developed as seminoma, as retained abdominal testes are wont to do.

Dr. Judson B. Gilbert, of Schenectady, N. Y., who has made a most thorough investigation of cancer in retained testicles,<sup>1</sup> informs me that he knows of no case similar to this in which the whole right genital anlage has failed to develop and then become a cancer.

#### SUMMARY

A case is reported of complete failure of the right genital anlage to develop, associated with immaturity of the left genital anlage. At the age of 47 the patient died with a retroperitoneal seminoma, apparently arising in the rest of undeveloped right genital anlage.

University of Chicago.

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1. Gilbert, J. B., and Hamilton, J. B.: *Surg., Gynec. & Obst.* **71**:731, 1940.

## UNIQUE CELL REST IN A UTERINE FIBROID

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A case is presented which is of unusual pathologic interest because of a rare and fascinating lesion that was found incidentally on microscopic study of the surgically removed uterus. The rarity would seem to be indisputable, for we have been unable to find in the literature any description of a comparable lesion in this particular situation, nor have we ever encountered a similar picture in any of our fairly extensive surgical or autopsy material. Its fascination lies in the attempt to account for its occurrence and localization on an embryologic basis.

### REPORT OF A CASE

A white woman of 40 years, married, was admitted to Temple University Hospital as a private patient of Dr. Harry A. Duncan, who made this brief clinical abstract available.

The patient had complained of progressive fatigability associated with a brownish vaginal discharge, which had been present for about a year. There had been no frank vaginal bleeding nor any other symptom immediately referable to the genital tract. The menstrual cycle had been regular, every twenty-eight days, the bleeding phase lasting for seven days. The last menstrual period had been approximately two weeks prior to admission.

Seventeen years previously the patient had undergone appendectomy, and twelve years before, the left fallopian tube was removed because of tubal pregnancy. Otherwise she had never had any serious illness. She has one child, 15 years of age, living and well.

Examination revealed nothing other than moderate enlargement of the uterus, thought to be due to multiple fibroids.

Abdominal hysterectomy, right salpingectomy and bilateral ovariectomy were performed following exploratory laparotomy. She made an uneventful postoperative recovery and was discharged cured.

*Gross Examination of Specimen.*—The specimen consisted of a uterus with the cervix attached, the right fallopian tube and the corresponding ovary. The uterus and cervix together measured 11 by 9 by 5 cm. opened. The contour of the uterus was distorted by several fibroids, the largest of which measured 4.5 cm. in diameter. This was subserous in location and situated on the lateral anterior wall of the fundus in close proximity to the left cornu. On the serosal surface there were several smaller similar-appearing masses, measuring about 3 to 5 mm. in diameter. On this same side there was a discolored soft spherical mass tightly adherent to the lateral aspect of the fundus of the uterus, which measured 4 cm. in diameter. This mass was multicystic; many of the cysts were filled with a thin chocolate-colored fluid material. The cyst linings were smooth. Grossly this tissue resembled ovary. There was no corresponding tube, as it had been removed for ectopic pregnancy at a previous operation, as specified in the case report. The cervix was firm and revealed small nabothian cysts. The endometrium was pale, velvety and smooth except for a submucous fibroid at the fundus. The

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From the Department of Pathology of the Temple University Hospital and School of Medicine.

right tube was grossly healthy. The corresponding ovary had an aberrant location, being situated at the midportion of the tube, to which it was tightly adherent. Cut sections showed moderate sclerosis, a small follicular cyst and a small corpus luteum.



Fig. 1.—Appearance of the cell rest under low power.

*Microscopic Study.*—There was an early premenstrual endometrium. The tube showed nothing of histologic significance. The right ovary was sclerotic and confirmed the gross findings of a small follicular cyst and of a small corpus luteum. The cystic mass described on the left side of the uterus proved to be ovarian tissue in which there were several cysts; the linings of the majority of these were

replaced by mononuclear phagocytes distended with old blood pigment, although one was covered by a layer of endometrial tissue.

Sections from the large subserous fibroid revealed that scattered throughout the fibromuscular tissue background there were nests and islands of cells with

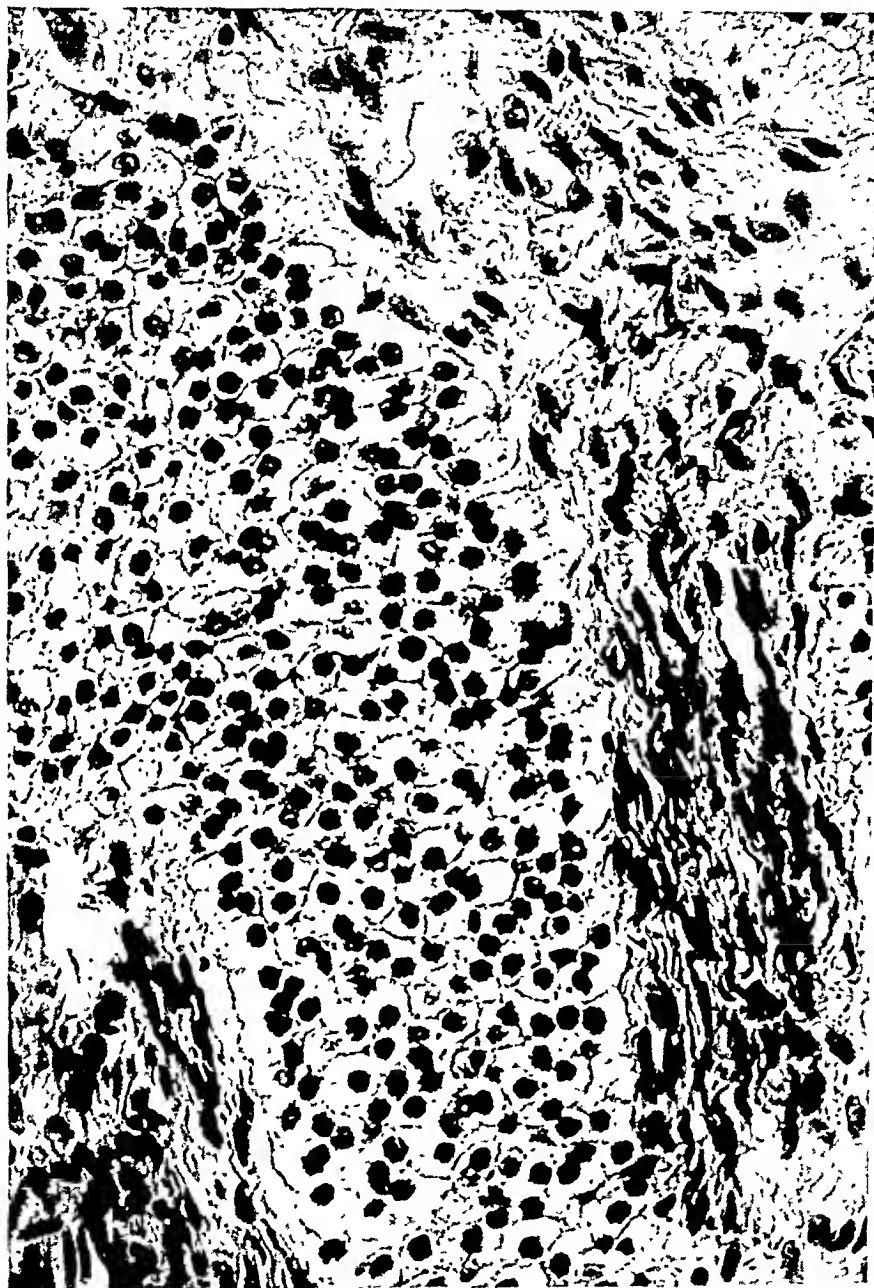


Fig. 2.—High power magnification of the cell rest.

clear cytoplasm and centrally placed, deep staining round nuclei. The peripheral cells in these nests and islands had a palisaded columnar appearance with the nuclei basally placed. There was neither cystic degeneration nor calcification. On cytologic grounds we concluded that these cells represented embryonal remnants and considered the possibility that they had their origin either in rests of adrenal cortex or in Walthard rests.

## COMMENT

Islands of adrenal cortex have been reported at the hilus of the ovary, and similar rests have been described even in the broad ligament.<sup>1</sup> So it is conceivable that they may be found in the serosa or the subserosa of the uterus. We were unable to do a fat stain, which would have been most helpful in accurately classifying these cells, because despite the fact that the specimen had been saved in solution of formaldehyde, almost serial section of the fibroid and of other areas throughout the uterus failed to reveal any similar-appearing cells. This would seem to indicate that these rests of cells were limited to a very small area.

In explaining the picture on the basis that the cells were derived from Walthard rests we must conjecture that these cells were present on the surface of the fundus uteri or that they were present in the broad ligament and that the tumor arose from the latter region with extension into the subserosa of the uterus. Such a theory is not too far fetched, because Walthard rests have been reported as low as the broad ligament, although they are more frequently and more commonly found in the ovary.<sup>2</sup> Masson and Van Gieson stains failed to be of any help to us in arriving at a definite conclusion. If this concept is true, we are dealing with a Brenner tumor which apparently arose in the broad ligament and grew into the subserosa of the uterus to appear grossly as a subserous fibroid.

The slides were submitted to several gynecologic pathologists for an expression of opinion regarding the nature of these cells. No unanimity of opinion was obtained, both the adrenal rest and the Walthard cell rest theories being supported. One pathologist finally concluded that the picture probably represented an unusual cancerous phenomenon in a fibroid. To the latter view we cannot subscribe.

## SUMMARY

A case in which peculiar unidentified rests of cells were found in what grossly appeared to be a subserous uterine fibroid is reported. We offer two possible explanations for their origin, namely, adrenal cortical cell inclusions or Walthard cell rests.

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2. Walthard, M.: *Ztschr. f. Geburtsh. u. Gynäk.* **49**:233, 1903. Akagi, Y.: *Arch. f. Gynäk.* **134**:390, 1928. Meyer, R.: *Zentralbl. f. Gynäk.* **56**:770, 1932. Meyer, R.: *Arch. f. Gynäk.* **148**:541, 1932. Novak, E., and Jones, H. W.: *Am. J. Obst. & Gynec.* **38**:872, 1939.



## OBSTRUCTIVE APPENDICITIS CAUSED BY A SPROUTING SEED

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AND

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In the most ancient writings on appendicitis<sup>1</sup> foreign bodies appear to be the usual cause of the disease. Mestivier, a French surgeon, performed the first modern operation for appendical abscess in 1759, and the subsequent autopsy showed that fatal peritonitis had been caused by a pin perforating the wall of the appendix. During the next fifty years four necropsies were reported in the French literature, and in each case a foreign body had perforated the appendical wall. Foreign bodies undoubtedly are found as frequently today as formerly, but owing to the large number of appendectomies done, the incidence appears to be relatively smaller. The following case seems to be of sufficient interest to warrant a report and short discussion.

### REPORT OF A CASE

A 22 year old white soldier was admitted to the Station Hospital, Fort Leonard Wood, Mo., at 1:30 a. m., Sept. 20, 1941, complaining of pain in the right lower abdominal quadrant of four hours' duration, associated with nausea, vomiting and a chill. At 7 p. m., three hours before the onset, he had received 1 cc. of typhoid vaccine (first injection) and 1 cc. of tetanus toxoid (second injection). At 10 p. m. he experienced a sudden pain in the right lower abdominal quadrant, and shortly thereafter, became nauseated and vomited. About 10:30 p. m. he had a chill and was seen by the dispensary medical officer who sent the patient to the hospital. The past history was significant in that there had been intermittent attacks of pain in the right lower abdominal quadrant several times a year for the past ten years. In August 1940 he had a severe attack, and operation was advised by his physician but was opposed by his family. After eight days in bed he recovered spontaneously. One month before admission a similar attack subsided in two days. On entry the temperature was 104 F., the pulse rate 120 and the respirations 20 per minute. There was slight generalized abdominal tenderness without point or rebound tenderness or muscle guard. The urinalysis disclosed no abnormalities. A blood count showed the hemoglobin content 100 per cent, erythrocytes 5,030,000 and leukocytes 15,300, with 90 per cent polymorphonuclear neutrophils, 7 per cent lymphocytes, 1 per cent basophils, 1 per cent eosinophils and 1 per cent monocytes. The patient was observed carefully during the night, and in the morning the temperature was 99 F., the pulse rate 94 and the respirations 22 per

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1. Collins, D. C.: *Ann. Surg.* 94:179, 1931.

minute. About 4 p. m. he had another chill, and the temperature rose to 101.2 F. An increase in abdominal pain accompanied this. At this time a blood count showed 26,500 leukocytes with 94 per cent polymorphonuclear neutrophils and 6 per cent lymphocytes. Shortly thereafter there was localization of the pain in the right lower abdominal quadrant with development of muscle guard, rebound tenderness and point tenderness over McBurney's area. At 7 p. m., with the patient under spinal anesthesia induced with 150 mg. of procaine hydrochloride, the peritoneal cavity was opened and the appendix was found lying over the brim of the pelvis. The surface vessels were congested, and the terminal centimeter of the appendix was firm and tense. The appendix was removed, and the stump was ligated and inverted. The temperature fell to 98.2 F. in the first six hours, and the highest postoperative temperature was 99 F. on the second day. A leukocyte count on the third day was 32,350, with 95 per cent polymorphonuclear neutrophils, 34 per cent lymphocytes and 2 per cent monocytes. On the fifth day the patient was out of bed and the skin superficially was well healed. The total leukocyte count at this time was 5,250 with a normal differential count. The further course in the hospital was uneventful, and the patient was discharged on the fourteenth postoperative day.



This photograph shows the appendical lumen obstructed by a sprouting seed. Note that the lumen distal to the point of obstruction is distended and that the rugations have disappeared. The wall is noticeably thinned. Proximal to the seed the lumen and wall are normal. Microscopic evidences of inflammation are limited to the area distal to the obstruction.

The appendix was 6 cm. long and 6 mm. in diameter, dilated to 7 mm. in diameter in the terminal 1.5 cm. The subserosal capillaries were congested, but there was no exudate. On longitudinal section the lumen was of normal caliber and the mucosa smooth to a point 1.8 cm. from the distal end, where a small flat seed was present. Just beyond this the lumen was obstructed by a round, moderately firm brown body, 2 by 2 by 2.5 mm. On section this object showed a dark brown cortical portion and a central portion occupied by a hypocotyl and root, coiled, 3 mm. in length. The mucosa of the dilated lumen distal to the obstruction was thin and smooth, and the lumen was filled with soft, brownish white material. The seed was identified as a piñon nut.

Microscopically, the mucosa and muscularis were relatively normal in the proximal part of the appendix, but in the dilated part the mucosa was flattened. Throughout the slightly edematous lymphoid tissue there were hyperplastic foci. Polymorphonuclear neutrophils were scattered in the muscularis of this part, singly

and in small groups. A few groups of fat cells intervened between the muscularis and the mucosa. The serosa was normal. The diagnosis was acute obstructive appendicitis.

One of us (W. F. B.) has previously reported on the findings in a series of appendixes.<sup>2</sup> The findings included foreign bodies other than fecaliths and pinworms in 2 per cent of the specimens. In 4 appendixes blueberry seeds were found, identified by competent authority. Three appendixes had small brown faceted stones in their lumens, and in 1 of the 3 the stones were identical with those removed from the gallbladder at the same operation. One appendix contained a spicule of bone which was piercing the wall and around which there was an inflammatory reaction, with pus in the lumen. Another appendical lumen contained a piece of keratinized material identified as finger nail. The final case was one in which a toothbrush bristle protruded from the side of a fecalith against the appendical wall. Royster<sup>3</sup> in his monograph stated that bristles, pins, hair, bone, seeds, shot, finger nails, teeth, screws, chewing gum, apple core and nut shells had been found in the appendical lumen. Some authors, of whom Burgess<sup>4</sup> is one, have simply stated that "many fruit seeds" were encountered in their series, but others, e. g., Matterstock<sup>5</sup> and Fitz,<sup>6</sup> place the incidence of foreign bodies in appendical lumens at 12 per cent. So far as is known, the case reported here is the only one in which a foreign body actually was growing in the appendical lumen.

The relationship of acute appendicitis to the lymphoid tissue swelling incident to typhoid inoculation has been made the subject of a separate paper in which 7 other cases are presented. In brief, it can be said that appendicitis in the vast majority of cases<sup>2</sup> is a phenomenon resulting from obstruction to the appendical lumen and that in the case reported here a seed partially occluded the lumen. Only the small amount of lymphoid swelling caused by the reaction to typhoid inoculation was needed to make the occlusion complete and then the chain of events incident to closed loop obstruction was in full swing. Similar lymphoid swelling has been reported to cause appendicitis in such diseases as measles,<sup>7</sup> mumps,<sup>8</sup> scarlet fever<sup>2</sup> and tonsillitis.<sup>9</sup>

#### SUMMARY

A case is presented in which acute suppurative appendicitis developed after typhoid inoculation because the appendical lumen was obstructed by a sprouting seed.

2. Bowers, W. F.: *Arch. Surg.* **39**:362, 1939.
3. Royster, H. A.: *Appendicitis*, New York, D. Appleton and Company, 1927.
4. Burgess, A. H.: *Brit. M. J.* **1**:415, 1912.
5. Matterstock, G. K.: *Perityphlitis*, in Gerhardts, C.: *Handbuch der Kinderkrankheiten*, Tübingen, H. Laupp, 1880, vol. 4, pt. 2, pp. 893-928.
6. Fitz, R. H.: *Am. J. M. Sc.* **92**:321, 1886.
7. Hudson, H. W., and Krakower, C.: *New England J. Med.* **215**:59, 1936.
8. Donnelly, J., and Oldham, J. B.: *Brit. M. J.* **1**:98, 1933.
9. Eguen, M.: *Tr. Sect. Laryng., Otol. & Rhin., A. M. A.*, 1932, p. 130.

# Forensic Medicine

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## CHANGES IN THE MAGNESIUM AND CHLORIDE CONTENTS OF BLOOD FROM DROWNING IN FRESH AND IN SEA WATER

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AND

ALAN R. MORITZ, M.D.

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The pathologic changes disclosed at autopsies on bodies recovered from water are frequently inconclusive in establishing the cause of death. In such instances the opinion that death has been caused by drowning is likely to be based in part on the fact that the body was found in water and in part on the fact that the postmortem examination failed to disclose any other cause of death. Obviously it would be desirable if the diagnosis of death by drowning could be supported by positive rather than negative evidence.

Gettler<sup>1</sup> called attention to the fact that diffusion between intra-alveolar water and capillary blood occurs after death by drowning and observed that the resulting changes in the blood are likely to be more pronounced in the left than in the right side of the heart. Thus after death by drowning in sea water the plasma chlorides may be disproportionately high in the left side of the heart, whereas after drowning in fresh water they are likely to be disproportionately low.

The inconstancy with which such changes are encountered after deaths known to have resulted from drowning, together with the fact that inequalities in the chloride content of samples of blood from the right and the left side of the heart are sometimes found in cases of death known to be due to causes other than drowning, led the authors to investigate certain aspects of the problem of agonal and postmortem migration of electrolytes under controlled experimental conditions.

### EXPERIMENTS

All experiments were made on dogs, which were first anesthetized by intravenous injection of soluble pentobarbital. In most instances the amount of soluble pentobarbital given was adequate to quiet the animal but not enough to abolish the palpebral reflexes.

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From the Department of Legal Medicine of Harvard Medical School and the Office of the State Pathologist, Massachusetts Department of Mental Health.

1. Gettler, A. O.: J. A. M. A. 77:1650, 1921.

Chloride was determined by the method of Schales and Schales<sup>2</sup> which consists in titration of a protein-free blood filtrate by a standard solution of mercuric sulfate, diphenylcarbasone being used as the indicator. With this method in this laboratory, added chloride has been recovered with an accuracy of 1 per cent. For the determination of magnesium a modification of the method of Fiske and Logan<sup>3</sup> was used: Following preliminary removal of calcium as calcium oxalate, the blood proteins are precipitated with trichloroacetic acid. The filtrate is concentrated and the magnesium precipitated as magnesium ammonium phosphate. With this method added magnesium has been recovered with an accuracy of 5 per cent.

Antemortem samples of blood were obtained by puncturing the heart through the intact thoracic wall. Postmortem samples<sup>4</sup> were taken from the right and left auricles after opening the chest. The openings in the thorax were made as small as possible and were kept closed during the intervals between sampling to prevent drying of the heart and lungs. In taking the postmortem samples the needle was introduced into the tip of each auricular appendage, and after each sampling the appendages were ligated proximal to the needle holes to prevent loss of serum.

Postmortem changes in the concentration of chlorides and magnesium in the blood were studied in four groups of dogs. The animals of the first group were put to death by clamping the trachea, and some of them were then allowed to remain in room atmosphere for as long as seventy-two hours. Those of the second group were put to death in the same manner but were then kept submerged in either fresh or sea water. The animals of the third group were drowned in a tank of fresh water, and the animals of the fourth group were drowned in sea water. After the animals of groups 3 and 4 were drowned, they were removed from the water and their tracheas were ligated to prevent escape of the inhaled water. They were kept at room temperature (about 75 F.) throughout the period of postmortem observation. Analysis of the sea water used in the experiments disclosed that it contained 1.75 per cent chlorides and 0.115 per cent magnesium. Analysis of the fresh water disclosed that it contained 3.2 parts per million chlorides and 1 part per million magnesium.

*Experiment 1.*—Six dogs were put to death by mechanical asphyxia (clamping of the trachea). Chloride determinations were made on 5 animals, and magnesium determinations were made on 4. Blood was taken at intervals from the right and left sides of the heart throughout the postmortem period. As indicated in tables 1 and 2, not all of the animals were kept at the same environmental temperature. Dog 1 was kept in a room maintained between 37 and 40 F.; dog 2, in a room maintained between 60 and 70 F., and dogs 3, 4, 5 and 6 in a room maintained between 75 and 85 F.

It may be seen in table 1 that the plasma chlorides in the heart's blood of these animals began to fall between six and twelve hours after death and continued to fall through a postmortem observation period of seventy-two hours. The rate of the loss of chlorides varied to some extent, and it seems likely that the rapidity with which

2. Schales, O., and Schales, S. S.: J. Biol. Chem. **140**:879, 1941.

3. Folin, O.: Laboratory Manual of Biological Chemistry, ed. 5, New York, D. Appleton-Century Company, Inc., 1934, p. 231.

4. It is obviously desirable to obtain comparable specimens from the two sides of the heart. Because of the artefact introduced by sedimentation and clotting, samples of plasma are likely to be more uniform than samples of whole blood.

the chlorides left the plasma increased with the rate at which postmortem autolytic and putrefactive changes developed.

In an animal kept in a cold room (37 to 40 F.) the chlorides in the right and left cardiac chambers were diminished by 25 and 15 per cent below the antemortem level, respectively, at the end of twenty-four hours. At the end of seventy-two hours the plasma chlorides in both sides of the heart had been reduced 27 per cent, or from 375 to 272 mg. per hundred cubic centimeters.

In the animals kept at higher postmortem temperatures the least reduction at the end of twenty-four hours was 27 per cent, or from 406 to 298 mg. per hundred cubic centimeters. The greatest was 45 per cent, or from 467 to 256 mg. per hundred cubic centimeters. At the end of forty-eight hours the percentile reductions in the plasma chlorides of these animals varied between 38 and 48, and at the end of seventy-two hours, between 48 and 51.

The fall in chlorides did not always occur at the same rate in both sides of the heart. In some it occurred more rapidly in the right side and in others more rapidly in the left. The greatest difference between samples from the right and left sides of the heart was observed in the case of dog 5 twelve hours after death and was 40 mg. per hundred cubic centimeters of plasma. Putrefaction had already begun, and both samples showed advanced hemolysis. It should be noted, however, that a difference of 35 mg. per hundred cubic centimeters of plasma was observed between the samples taken from the right and left sides of the heart of dog 1 twenty-four hours after death. In this animal there was neither evidence of putrefaction nor a significant amount of hemolysis to account for the discrepancy.

It appears from the data shown in table 1 that a reduction in plasma chloride of as much as 103 mg. per hundred cubic centimeters, or 27 per cent, below the antemortem level in a nonputrid animal and of 240 mg. per hundred cubic centimeters, or 51 per cent, below the antemortem level in a putrid animal may represent normal postmortem changes. It also appears that differences as great as 40 mg. of chloride per hundred cubic centimeters may be encountered in post-mortem samples of plasma simultaneously obtained from the two sides of a dog's heart.

In table 2 it is shown that the concentration of magnesium in the plasma increased after death. In 2 animals from which samples were taken relatively soon after death the elevation was apparent within six hours.

In an animal kept in a cold room (37 to 40 F.) the plasma magnesium in the right and left chambers was increased by 38 per cent above the antemortem level, or from 2.3 to 3.7 mg. per hundred cubic centimeters by the end of the first twenty-four hours. At the end of seventy-two hours the increase was by 157 and 191 per cent, or to 5.9 and 6.7 mg. per hundred cubic centimeters, respectively, in the right and left chambers.

In 4 animals kept at higher postmortem temperatures the plasma magnesium of the cardiac blood was more than doubled at the end of twenty-four hours. At the end of forty-eight hours it was increased to between three and six times its antemortem level. In dog 3 the plasma magnesium reached 20.8 mg. per hundred cubic centimeters, compared with an antemortem level of 2.4 mg.

As was observed in the case of chlorides, the magnesium levels did not always change at the same rate in the two sides of the heart. In some instances that in the right rose more rapidly, and in others, that in the left. The greatest difference observed at any time in 11 pairs of postmortem samples was 0.9 mg. per hundred cubic centimeters.

It appears from the data shown in table 2 that an increase in the plasma magnesium of as much as 4.4 mg. per hundred cubic centimeters in a nonputrid

TABLE 1.—*Postmortem Changes in the Concentration of Plasma Chloride in the Right and Left Sides of the Hearts of Dogs Killed by Tracheal Compression*

Time	Dog 1			Dog 2			Dog 3			Dog 4			Dog 5		
	Postmortem			Postmortem			Postmortem			Postmortem			Postmortem		
	Room Temperature 37 to 40 F.			Room Temperature 60 to 70 F.			Room Temperature 75 to 85 F.			Room Temperature 75 to 85 F.			Room Temperature 75 to 85 F.		
	Side of Heart			Side of Heart			Side of Heart			Side of Heart			Side of Heart		
	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.
Ante mortem.....	375	375	..	407	467	..	403	406	..	410	410	..	413	413	..
Post mortem															
15 minutes.....	...	...	..	...	...	..	...	410	..	410	429	13	416	429	13
6 hours.....	...	...	..	...	...	..	406	410	4	403	422	19	...	...	..
12 hours.....	...	...	..	...	...	..	381	...	..	...	...	..	...	...	..
24 hours.....	282	317	35	256	278	22	298	302	4	276	276	0	314	354	40
48 hours.....	...	...	..	253	243	10	...	...	..	262	256	6	276	305	29
72 hours.....	272	272	0	232	227	5	231	...	..	...	...	..	...	...	..
							212	...	..	...	...	..	...	...	..

TABLE 2.—*Postmortem Changes in the Concentration of Plasma Magnesium in the Right and Left Sides of the Hearts of Dogs Killed by Tracheal Compression*

Time	Dog 1			Dog 2			Dog 3			Dog 6		
	Postmortem			Postmortem			Postmortem			Postmortem		
	Room Temperature 37 to 40 F.			Room Temperature 60 to 70 F.			Room Temperature 75 to 85 F.			Room Temperature 75 to 85 F.		
	Side of Heart			Side of Heart			Side of Heart			Side of Heart		
	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Difference, Mg.
Ante mortem.....	2.3	2.3	...	2.3	2.3	...	2.4	2.4	...	2.4	2.4	...
Post mortem												
15 minutes.....	...	...	...	...	...	...	2.6	2.3	0.3	2.3	2.6	0.3
6 hours.....	...	...	...	...	...	...	3.4	3.3	0.1	3.3	3.4	0.1
12 hours.....	...	...	...	...	...	...	5.8	5.2	0.6	5.6	5.8	0.2
24 hours.....	3.7	3.7	0.0	6.8	5.9	0.9	7.3	...	...	7.3	...	...
48 hours.....	...	...	...	8.6	8.5	0.1	16.0	...	...	16.0	...	...
72 hours.....	5.9	6.7	0.8	10.1	10.2	0.2	20.8	...	...	...	...	...

animal and one of as much as 18.4 mg. per hundred cubic centimeters in a putrid animal may result from postmortem change. It also appears that a difference as great as 0.9 mg. of magnesium per hundred cubic centimeters of plasma may be encountered in postmortem samples simultaneously obtained from the two sides of a dog's heart.

**EXPERIMENT 2.**—This experiment was undertaken to learn whether or not post-mortem submersion of an animal already dead of causes other than drowning will modify the rate and the character of the chemical changes that normally take place in the plasma after death.

Two lightly anesthetized dogs were killed by compression of the trachea. One (dog 7) was then submerged for seventy-two hours in fresh water, and the other (dog 8) was submerged for forty-eight hours in sea water.

The antemortem plasma chloride content of a sample taken from the heart of dog 7 was 432 mg. per hundred cubic centimeters. After seventy-two hours' submersion in fresh water the plasma chloride levels in the right and left sides of the heart had dropped to 266 and 278, respectively. The plasma magnesium concentration of an antemortem sample taken from this dog was 1.9 mg. per hundred cubic centimeters. After submersion of the dog in fresh water for seventy-two hours the plasma magnesium had risen to 8.0 mg. per hundred cubic centimeters in the right side of the heart and to 7.7 mg. per hundred cubic centimeters in the left.

The antemortem value of the plasma chloride of a sample taken from the heart of dog 8 was 448 mg. per hundred cubic centimeters. After forty-eight hours' submersion in sea water the plasma chloride levels in the right and left sides of the heart had dropped to 368 and 328 mg., respectively. The concentration of plasma magnesium in an antemortem sample taken from this dog was 2.1 mg. per hundred cubic centimeters. After forty-eight hours' submersion in sea water the concentration in the right side of the heart had increased to 6.6 mg. per hundred cubic centimeters and that in the left to 6.3 mg. per hundred cubic centimeters.

Although both the fresh and the sea water tanks remained at approximately the same temperature (60 to 70 F.), there was considerable difference in the rate at which putrefaction developed in the submerged animals. There was little evidence of putrefactive change in dog 8 after forty-eight hours' submersion in sea water, whereas putrefactive changes were well developed in dog 7 at the end of seventy-two hours. It seems likely that the relatively small loss of chlorides from the heart's blood in dog 8 (82 mg. from the right and 122 mg. from the left) as compared with control dog 2 (see table 1), which was kept in the air at approximately the same temperature, could be better accounted for by the fact that the latter putrefied more rapidly than the former than by the assumption that sea water had entered the lungs of dog 8 after death. There was neither chemical nor pathologic evidence that water had entered the lungs of either of the animals that were submerged after death.

**EXPERIMENT 3.**—Two dogs were drowned by submersion in a tank of fresh water. The first (dog 9) was lightly anesthetized and struggled for about a minute immediately after submersion, during which time the animal held its breath. Immediately thereafter it lapped water vigorously for a minute or two and then vomited. The dog then began to inhale water, at first slowly and later rapidly. Toward the end the respiratory movements became irregular and spasmodic. All respiratory activity ceased at the end of about four minutes, and the heart continued to beat for several minutes thereafter. The second animal (dog 10) was more deeply anesthetized and did not struggle after being placed in the tank. It did not lap water, and all respiratory movement stopped after about two minutes' submersion. The heart beat was not perceptible after the cessation of respiratory movement.



Each dog after respiration had ceased was placed on the operating table and kept at room temperature (about 75 F.) throughout the duration of the experiment. The plasma chloride determinations for the two animals are shown in table 3.

Within fifteen minutes after death the plasma chloride concentrations in the right and left sides of the heart of dog 9 were reduced by 41 and 70 per cent, respectively. The difference between the chloride concentrations in the two sides of the heart at this time was 134 mg. The chloride concentration in the right side of the heart continued to fall whereas that in the left side remained relatively stationary. At the end of seventy-two hours the chloride had dropped 62 per cent below the antemortem level in the right side of the heart and 69 per cent in the left. During the first twenty-four hours after death the difference between the chloride concentrations in the right and left sides of the heart was in excess of 75 mg. per hundred cubic centimeters, which was greater than had been observed in any of the animals in the control series. After putrefaction was established (forty-eight hours post mortem) the differences in chloride concentration were diminished to a point at which they were no greater than some of the differences that were observed in control animals.

TABLE 3.—*Changes in the Concentration of Plasma Chlorides in the Right and Left Sides of the Hearts of Two Dogs Drowned in Fresh Water*

Time	Dog 9			Dog 10		
	Side of Heart		Differ- ence, Mg.	Side of Heart		Differ- ence, Mg.
	Right, Mg. per 100 Cc.	Left, Mg. per 100 Cc.		Right, Mg. per 100 Cc.	Left, Mg. per 100 Cc.	
Ante mortem.....	454	454	0	397	397	0
Post mortem						
15 minutes.....	269	135	134	342	332	10
6 hours.....	256	...	106	294	307	13
12 hours.....	256	144	112	...	...	...
24 hours.....	227	150	77	269	243	26
48 hours.....	...	...	...	...	...	...
72 hours.....	173	141	32	...	...	...

Dog 10, which was more deeply anesthetized than dog 9, died without a struggle and soon after being submerged. This may account for the fact that there was less evidence of plasma dilution. Within fifteen minutes after death the chlorides in the right side of the heart had dropped 55 mg. and those in the left side 45 mg. In contrast it should be noted that in the control animals (see table 1) there was no significant change in the chloride concentrations of samples taken fifteen minutes after death. At the end of six hours the plasma chlorides of dog 10 were reduced by 26 per cent in the right side of the heart and by 23 per cent in the left side. No such dilution had been observed in control animals within the first six hours after death. At no time in dog 10 were the differences observed between the concentrations of chlorides in the right and left sides of the heart greater than some of those that were observed in control animals.

In both of the animals drowned in fresh water the postmortem reduction in plasma chloride concentrations occurred earlier than it did in the animals of the control series. In one of the drowned animals the difference between the chloride concentrations in the right and left chambers caused by disproportionate dilution of the blood in the left side was significantly greater during the first twenty-four hours after death than that in the control animals. In both drowned animals there was marked hemolysis of the blood in the left auricle as early as fifteen minutes after death, whereas in the control series a significant degree of hemolysis (5 per cent hemoglobin in the plasma) did not develop until six hours or more after death.

EXPERIMENT 4.—Three dogs were anesthetized lightly and were then drowned by submersion in sea water. In each the reaction to submersion was similar to that observed in dog 9. After all respiratory movements had ceased, the animals were removed from the water and samples of blood for chloride and magnesium analysis were taken from the right and left sides of the hearts at the intervals indicated in tables 4 and 5.

It may be seen that during the first twenty-four hours after death the differences between both the chloride and the magnesium concentrations in the two sides of the heart were greater than had been observed at any time in animals of the control

TABLE 4.—*Changes in the Concentration of Plasma Chlorides in the Right and Left Sides of the Hearts of Dogs Drowned in Sea Water*

Time	Dog 11			Dog 12			Dog 13		
	Side of Heart			Side of Heart			Side of Heart		
	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.
Ante mortem.....	438	438	..	435	435	..	442	442	..
Post mortem									
15 minutes.....	483	537	54	...	...	..	...	...	..
6 hours.....	403	570	167	...	...	..	...	...	..
12 hours.....	397	601	204	493	541	48	467	547	80
24 hours.....	...	...	...	390	480	90	339	438	99
48 hours.....	386	342	6	381	422	41	330	368	38
72 hours.....	...	...	...	384	406	22	323	342	19

TABLE 5.—*Changes in the Concentration of Plasma Magnesium in the Right and Left Sides of the Hearts of Dogs Drowned in Sea Water*

Time	Dog 11			Dog 12			Dog 13		
	Side of Heart			Side of Heart			Side of Heart		
	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.	Right, Mg. per 100 Ce.	Left, Mg. per 100 Ce.	Differ- ence, Mg.
Ante mortem.....	2.5	2.5	....	2.5	2.5	....	1.9	1.9	....
Post mortem									
15 minutes.....	5.2	15.3	10.1	....	....	....	....	....	....
6 hours.....	...	25.0	...	...	...	...	...	...	...
12 hours.....	10.6	29.5	18.9	9.7	22.7	13.0	9.1	19.3	10.2
24 hours.....	...	...	...	10.6	19.4	8.8	12.4	18.0	5.6
48 hours.....	13.0	20.6	7.6	14.7	16.5	1.8	14.7	15.0	0.3
72 hours.....	....	....	....	20.4	19.6	0.8	22.5	22.0	0.5

series. The least difference between chloride concentrations in the right and left chambers within the first twenty-four hours after death was 48 mg. and the greatest 204 mg. The least difference between magnesium concentrations was 5.6 mg. and the greatest 18.9 mg. In all observations made within twenty-four hours after death the concentrations of chlorides and magnesium in the left were significantly greater than those in the right side of the heart. After twenty-four hours the differences in both the chloride and the magnesium concentrations between the two sides of the heart became less pronounced.

Even after putrefaction was fully developed in the animals drowned in salt water the plasma chlorides did not fall below 300 mg. per hundred cubic centimeters, whereas the chloride levels in putrefied animals of the control series were ordinarily much lower. Analysis of the gastric contents of animals drowned in sea water disclosed magnesium concentrations that varied between 0.075 and 0.100 per cent.

## COMMENT

It has been observed in dogs that striking changes take place in the chloride and the magnesium content of the heart's blood after death. A decrease in chlorides and an increase in magnesium are apparent within six to twelve hours post mortem and become progressively greater during the next two or three days. By the end of seventy-two hours the chlorides may diminish by as much as 50 per cent and the magnesium may increase by eightfold or more. Although the rate at which these changes occur is affected by the environmental temperature, they are not completely inhibited by room temperatures as low as 40 F. The results obtained from analysis of human blood samples indicate that similar postmortem changes may occur in man.

It was observed that the postmortem chemical changes do not always progress at the same rate in the two sides of the heart. In some animals they occur more rapidly in the right side and in others in the left.

The differences between the concentrations of plasma chloride in the right and left sides of the heart as determined on 16 pairs of postmortem samples from control animals (experiments 1 and 2) ranged between zero and 40 mg. per hundred cubic centimeters. The distribution of the differences followed a normal curve, and statistical analysis disclosed the standard deviation of the differences to be 20.6. It would appear that a difference in excess of 60 mg. (approximately three times the standard deviation of the differences observed in control animals) would constitute evidence that some unusual unilateral alteration in electrolyte concentration had taken place.

The differences between the concentrations of plasma magnesium in the right and left sides of the heart as determined on 13 pairs of post-mortem samples from control animals varied between zero and 0.9 mg. per hundred cubic centimeters. These differences also appeared to follow a normal distribution curve, and their standard deviation was 0.42. As with the differences in chloride concentration it is felt that a difference in the concentrations of plasma magnesium in the two sides of the heart in excess of three times the standard deviation (about 1.25 mg. per hundred cubic centimeters) should be regarded as evidence of abnormal unilateral concentration or dilution.

In observations on human material chloride differences as great as 50 mg. and magnesium differences as great as 1.0 mg. per hundred cubic centimeter of plasma were encountered in postmortem samples taken from the right and left sides of the heart of persons dead of causes other than drowning.

Within fifteen minutes after drowning in fresh water the concentrations of plasma chlorides in both sides of the hearts of the two animals used in experiment 3 were found to be reduced to levels not ordinarily encountered in any of the control animals until much later in the post-

mortem period. The generalized dilution of blood of these animals was interpreted as evidence that considerable antemortem diffusion of electrolytes had occurred between the pulmonary capillaries and the fluid that had been aspirated into the lungs. Similar generalized changes in the electrolyte concentration of the blood have been observed in human subjects after death by drowning.

In one of the 2 animals that had been drowned in fresh water the chlorides in the left side of the heart during the first twenty-four hours after death were found to be much lower than those in the right. The difference between the chlorides in the two sides of the heart of this animal was significantly greater than the greatest difference observed in the control series and indicates that disproportionate dilution of the blood in the left side of the heart had occurred. It was observed, however, that the difference became less as the hours passed and after the development of putrefaction became so small as to lack significance.

In the other animal drowned in fresh water disproportionate dilution of electrolytes in the left side of the heart was not observed even in the samples which were taken early in the postmortem period. It is perhaps significant that this animal was more deeply anesthetized than any of the other animals and died with relatively little struggle and within two minutes after submersion. Although the reason why disproportionate dilution of the blood in the left side of the heart occurred in one animal and not in the other is obscure, it seems likely that the manner in which the terminal heart failure occurred may have been an important factor. If the agonal circulatory collapse is gradual rather than abrupt, it is entirely possible that even though the terminal ventricular contractions propel a certain amount of chemically altered blood out of the pulmonary vessels into and through the left side of the heart, so much of it is pooled in the dilated vessels of the systemic circulation that significant terminal chemical differences of blood are established between the right and left sides of the heart. If, however, the agonal circulatory collapse is sudden, so that blood is circulating actively through both the pulmonary and the systemic circuits until the moment of asystole, the chemical differences of the blood contained in the two sides of the heart might well be so slight as to be imperceptible. If the foregoing hypothesis is correct in the case of the dog, it may also serve to explain the absence of disproportionate electrolyte concentration that is sometimes encountered in persons who have died by drowning.

A striking change observed in both the animals that were drowned in fresh water was early hemolysis of the blood, particularly of that in the left side of the heart. Samples of plasma withdrawn as early as fifteen minutes after death contained in excess of 5 per cent hemoglobin, whereas in the control animals a comparable degree of hemolysis was not observed until many hours had elapsed.

Immediately after death from drowning in salt water it was observed that the chlorides and the magnesium were elevated in both sides of the heart. Chloride levels observed during the first six hours after drowning in salt water were higher than the levels observed at any time in the control animals. Magnesium levels during the first twelve hours after drowning in sea water were consistently higher than those observed in control animals prior to the development of putrefaction. The increase in chlorides and magnesium was greater in the left than in the right side of the heart in all 3 animals drowned in sea water. Even after the development of putrefaction the plasma chlorides of these animals failed to fall below 300 mg. per hundred cubic centimeters, whereas in the control series chloride levels of 300 mg. were invariably present after putrefaction had developed.

Additional observations on animals dead of drowning in sea water included that of a relatively high (0.075 to 0.100 per cent) concentration of magnesium in the gastric contents and that of delay in postmortem hemolysis of the heart's blood.

There was neither pathologic nor chemical evidence that significant concentration or dilution of the heart's blood occurs when an animal dead of causes other than drowning is submerged in salt or fresh water.

#### SUMMARY

In both dogs and man a progressive loss of plasma chlorides accompanied by an increase in plasma magnesium represents a normal postmortem phenomenon.

These changes do not always occur at the same rate in the two sides of the heart. In dogs differences in chlorides as great as 40 mg. and in magnesium as great as 0.9 mg. per hundred cubic centimeters of plasma may be encountered within twenty-four hours after death. Comparable differences have been observed in man.

After drowning in fresh water not only may the plasma chlorides be reduced in both sides of the heart to levels not ordinarily encountered in comparable samples from control subjects but the reduction may be significantly greater in the left than in the right side.

After drowning in sea water the chlorides and the magnesium are increased in both sides of the heart to levels not ordinarily encountered in control subjects and the increases are likely to be significantly greater in the left than in the right side.

The agonal and early postmortem differences between the chemical constitution of the blood on the left and that of the blood on the right side of the heart which may exist after drowning in either fresh or sea water tend to disappear as putrefaction progresses.

## General Reviews

### LEPROSY FROM THE HISTOLOGIC POINT OF VIEW

*"quod ante haec dua annorum milia vitae origines in  
ipsis fortibus corrupuit"* . . . —Ihm.

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Danielssen and Boeck, in 1847, illustrated histologic studies of leprosy for the first time. They described oblong cells comprising the nodules, larger than the usual inflammatory cells. Simon, in 1848, illustrated a section through a nodule, describing its cellular and connective tissue structure. Although Danielssen and Boeck described yellowish brown granules in the lesions, they inclined toward the idea of leprosy as a hereditary rather than as an infectious disease. There are a few other descriptions in the 1850's and early 1860's, such as that of Kobner, who spoke of the infiltrations as small in the deeper layers of the skin, enlarging near the surface and becoming continuous in the papillary layer, ". . . distributed about vessels as well as sebaceous or sweat glands."

Among the numerous descriptions of leprous lesions that followed during the period preceding 1879 (when Neisser demonstrated the acid-fastness of Hansen's bacillus), that of Virchow is one of the more intelligible:

. . . In connection with the cells, I note that at the height of their development they consist of round, pale, finely acinous, easily perishable elements with at most a moderately large and at the same time granular nucleus with nucleoli. In the fresh state there is a particularly notable peculiarity, namely, their marked tendency to form a sort of vacuole, apparently from taking up water, so that they acquire under the circumstances a wholly physaliferous<sup>1</sup> appearance.

✓ Virchow noted many features of the nodule—the flattened epidermis, the loss of hairs, the involvement of arrectores pilorum muscles, the accumulation of fat as the lesions aged and the development of new processes above the old. He derived the lepra cells from connective tissue cells, in which he found nuclear divisions and described fibrous nerves.

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From the Division of Pathology, National Institute of Health, United States Public Health Service.

1. From *φυσαλλίς*, a little bladder, hence a bubble, so that the word means foamy.

Gerhard Armauer Hansen published his conviction of the etiologic importance of the "brown granules" in 1874. He adopted the expression from Danielssen and had used it previously to this time but not with such certainty. That Hansen recognized the individual bacilli forming the masses as the essential units is clear from his descriptions:

. . . The cells, but not the brown elements, swell considerably in water, and if one examines them under a strong lens, one discovers in many of them, besides granules, also rod-shaped bodies. At times one will find the rods together in bunches crossing each other at very sharp angles.

Neisser's demonstration brought the incunabular period of the concept of leprosy to a close. It was followed by a paper by Hansen, giving many details of the relation of the bacilli to cells and tissues, and by another more extensive descriptive article by Neisser, in which he stated that Hansen had rushed into print to enjoy precedence to which he was not entitled. During the last two decades of the nineteenth century a large number of articles appeared on the anatomy and the histology of leprosy, with some description of every bare anatomic detail. Hansen in 1901 exclaimed that the literature of leprosy was "already vast enough," but with the exception of the period of the war it has increased steadily and voluminously.

#### TERMINOLOGY

Danielssen and Boeck divided leprosy into two forms, the tubercular and the neural. A considerable evolution in terms has taken place in ninety-five years. Although "tubercular" is still used at times, it has given way in many instances to "cutaneous," "granulomatous," "nodular" and "lepromatous"; the last is much in use today. Tubercular leprosy is not to be confused with tuberculoid leprosy (described in later paragraphs). At the other extreme, the term "neural leprosy" has had a stormy career. The discovery has been made repeatedly that the milder forms of leprosy are ipso facto those with few bacilli. The term "lepid" has been much used to signify a macule without bacilli or with extremely few organisms, and "neurolepid" to imply a relation to cases in which nerve changes are outstanding. The broad, flat lesions led Hansen and Looft to the term "maculoanesthetic leprosy" as a partial substitute for "neural leprosy," and the expression "mixed leprosy" is an acknowledgment that many cases present macules, anesthetic areas, nodules and other changes.

#### PRIMARY AND TRANSITIONAL STAGES

Probably the first important studies of leprosy in its early stages were those begun by Gomez, Avellana Basa and Nicolas in the Philippine Islands. They followed the children born to institutionalized lepers to discover early lesions. These have been supplemented by additional studies by Lara and de Vera, Rodriguez (1926, 1932), Chiyuto, and

Nolasco and Lara. Other, not wholly comparable studies of children have been instituted by Cochrane and Rajogaplan in India, Wayson in Hawaii, de Souza Araujo in Columbia and Rodrigues in Brazil.

The respiratory and gastrointestinal tracts have been considered by Lowe and Muir to be possibly of no importance compared with the skin as a portal of entry of the bacillus, and the question of a primary lesion of the skin akin to the Ghon tubercle or the chancre of syphilis has often been raised. An interesting commentary on this was offered by Nolasco and Lara in their recent description of a case of infantile leprosy, with a review of other published cases thereof. The upper air passages, which are so regularly the seats of leprous infection, offer an easy and simple explanation, which proves on the whole unsatisfactory (Solis and Wade). Nolasco and Lara suggested that previous lesions of the skin, often due to scabies, offer a site for the development of first lesions, possibly affording a portal of entry for the organisms. Of more direct interest is their finding that in 11 of 14 cases of genuinely early involvement the disease was tuberculoid in histologic character, i. e., showing principally tubercle formations of epithelioid and giant cells.

The first case of tuberculoid leprosy described was that of Montgomery, who saw in a lesion of the face giant cells which were "too circular" and "surrounded by one row, usually complete, of round nuclei." He interpreted these as hyalin-filled capillaries cut in cross section, the nuclei representing hyperplastic endothelium. Nonetheless, he made a remarkably apocryphal statement concerning sections that were examined fruitlessly for bacilli: "This is exactly what was to have been expected." Jadassohn presented a clearcut case of tuberculoid leprosy, and Arning described a nerve abscess with tuberculoid and caseous changes. Jadassohn's case showed epithelioid cell tubercles and some caseation. Blaschko and Glück told of having seen somewhat similar changes. Additional examples followed through the years, from Hodara, Tshlenow, Brutzer, Klingmüller (1900), Tièche, Merian, Kedrowsky, E. Hoffmann, Unna Jr., Bruusgaard, Darier, Pautrier and Boez, Tebbut and Molesworth, Balina and Basombrio, and Tisseuil. But the modern trend began with the publication by Wade and Pineda of the results of their survey of "negative lepers" at Culion, Philippine Islands, among whom they found an astonishingly large number showing this type of process.

Wade distinguished two forms of tuberculoid leprosy, major and minor. Marked clinical differences, with the usual presence of bacilli in the major and their absence from the minor form, serve to distinguish them. Concerning lesions of the major form, Wade and Rodriguez (1939) wrote:

. . . On the whole they differ rather widely from the ordinary chronic tuberculoid leprids, in which the granulomatous foci are more or less distinct. . . . For



the most part the granuloma is more diffuse and less differentiated, the component cells not distinctly of the epithelioid form or if so not aggregated in characteristic foci; giant cells are not numerous or conspicuous or typical.

Although Wade saw such lesions regress completely in 1 case, he and Rodriguez (1940), des Essarts and Lefrou (1936) and Schujman described the transition of this form into the frankly nodular variety. I have seen a few cases in which the changes were mixtures of tuberculoid and lepromatous reactions and a case in which the cells were curiously neither epithelioid nor vacuolated but hybrids with many bacilli. It seems clear that transitions between major tuberculoid and nodular leprosy must not be altogether rare.

Most of the writings, old and new, are of the minor tuberculoid lesions, which are meant by the unspecified use of the word. Brutzer described the cells forming tubercles particularly about capillaries, but it has since been recognized that they occur also in much the same positions as the bacillus-containing foci of macular or nodular lesions, closely related to the appendages of the skin.

The foci occur often in a layer close to the surface, perhaps, as Klingmüller (1927) said, because "the papillary area in leprosy as in tuberculosis provides a site for the removal of bacilli from the blood stream." Wade noticed that the cells of the tubercles commonly lie in actual contact with the cells of the basal layer of the epidermis, with fraying of the cytoplasm of the latter. A long series of incompletely separate foci may extend along the surface, flattening the epidermis only over small areas, thus giving rise to the clinical appearance of groups of minute papules.

The actual arrangements and developments of the epithelioid cells, giant cells and lymphocytes which form the bulk of the foci are quite variable and probably dependent on the status of the disease as a whole. The giant cells may be as large as those in tuberculosis but rarely show the crescentic arrangement of polarized nuclei. More often they are round, with sharply limited cytoplasm, which in the center shows fine granules against a nearly clear background. The nuclei lie at the periphery, sometimes with spherical regularity. Variations in size and shape are countless; the giant cells may resemble foreign body giant cells, and occasionally contain foreign bodies. Des Essarts and Lefrou (1934) distinguished the character of the tubercles of leprosy from those of tuberculosis histologically as showing much more uniform tinctorial properties but much more irregularity otherwise. The leprosy ones affect the epidermis less, are more vascular and contain a greater variety of cells. Old leprosy tubercles are commonly sharply outlined histologically by a thin fibrous capsule, but in earlier lesions the peripheral lymphocytes and histiocytes mingle loosely with the neighboring collagen fibers.

In sections the tuberculoid foci may appear discrete, but whether in serial sections they would be is doubtful, as foci often originate in accumulations of lymphocytes and other cells along blood vessels and nerves, and many of the lesser lesions appear as minute groups of epithelioid cells without giant cells in perivascular cuffs of ordinary chronic inflammatory cells. With regression of the lesions, atrophic changes take place in the tubercles to the point of complete disappearance. An extensive lesion may leave as a residue only the collapsed framework of the granuloma.

Tisseuil (1930) suggested that the tuberculoid foci of epithelioid cells (without giant cells) represent intermediate stages of development, and Rabello Jr. issued (1937) another more elaborate classification of tuberculoid lesions into (1) pretuberculoid, (2) sarcoid, (3) lupoid or follicular, with bacilli present, and (4) colliquative with caseation. Portugal adopted this classification, in which the sarcoid would correspond to Wade's minor tuberculoid, the lupoid to the major and the colliquative to the nerve abscess or bullous ulcerative (lazarine) lesion.

Caseation, necrosis or signs of simple inflammation are usually absent from the lesions of tuberculoid leprosy. There is another variety, however, which appears rarely, in which widespread ulceration of the skin over the lesions follows the formation of bullae. Cases of this type, as described by Nicolas, Gaté and Ravault, Ryrie, Lowe and Chatterji, Rodriguez (1935) and Wade and Rodriguez (1940), for which the term "lazarine leprosy" has been used, seem to be cases of Wade's major tuberculoid variety.

The rarity of bacilli in the lesions of minor tuberculoid leprosy is a consistent feature. When bacilli are found, they are in relation to the epithelioid cell foci, rarely in giant cells, most often within outer marginal epithelioid cells or in undifferentiated wandering cells at the margins of epithelioid cell foci, singly rather than in groups. However, if a group of bacilli is formed, the cell becomes vacuolated and identical with the lepra cell. The epithelioid cells may contain a diffuse fine deposit of lipid. Among different examples of tuberculoid lesions it is probable that some can be found showing all stages of development from minor to major forms, as well as regressive lesions in which no bacilli can be found in spite of exhaustive search, for which Merian recommended the use of antiformin (a strongly alkaline solution of sodium hypochlorite) and in the study of which fluorescent microscopy might prove to have some value.

Wade and also Lowe inclined to the belief that all macular leprosy is basically tuberculoid. On the other hand, Portugal expressed the opinion that a distinction should be maintained between "simple" and tuberculoid leprids. Ermakova found the lesions in most of his cases

to be simple leprids without tuberculoid changes. Ota and Sato (1937), expressed the opinion that tuberculoid leprosy should not be considered a special form of the disease. Montel and Bablet preferred the term "tuberculids" of leprosy. Rabello Jr. expressed the belief that tuberculoid leprosy represents a transition from the neural to the nodular form in the sense that it is a temporary stage, which shows differences in development from minute groups of epithelioid cells to the fully formed tubercles (Parmakson).

Hughes summed up the general belief that "tuberculoid leprosy is the natural evolution of immunity in the disease" and that it represents the peak of the local response.

There is also the peculiar problem of Boeck's sarcoid and the marked similarity of that lesion histologically to fully developed tuberculoid leprosy without bacilli (Filho; Reenstierna; de Souza and Adjuto). Lisi and Sebastiani used the expression "sarcoids of leprosy," and Mottat emphasized the similarity of tuberculoid lesions to other cutaneous sarcoids. Reenstierna remarked that a diagnosis of Boeck's sarcoid is not likely to be made in communities with a high rate of leprosy and pointed to the lack of consideration of leprosy in cases of this disease in zones where the little leprosy present might be predominantly of the more benign tuberculoid type.

#### TYPES OF LESIONS

*Macules and Leprids.*—Although every possible variation in histologic change is seen in leprosy, and although one may subscribe to the dictum of Leloir (1885), "Il n'y a qu'une lèpre, à l'évolution variable" (There is only one leprosy variable in its evolution), it is necessary to point to one phenomenon which distinguishes the spreading macule from the nodule. The macule, irrespective of its histologic appearance, is commonly multiple, annular and intermittently active at the borders only. As it advances there is left a central area which is anesthetic and partly depigmented and which shows histologically only banal cellular changes. The central part of the macule is resistant to further macular spread. But this area is as susceptible as any other to the development of a lepromatous lesion. Another ancient truism emphasizes the perplexity: The histologic changes of the young nodule are identical with those of the active nontuberculoid macule. Piscane said that the differentiation of neural from nodular lesions is quantitative, not qualitative; or there is Darier's statement (1897): "Par un série insensible de gradations elles se rapprochent des léprômes en nappe" (By an imperceptible series of gradations they are brought nearer to diffuse lepromas). Klingmüller (1902), studying reports of multiple biopsies in 3 cases of macular leprosy, stressed the numbers of bacilli present as an important factor

in the variability of the picture and observed the spread of macules irrespective of the nodular formations.

[The earliest or slightest lesion consists of cells about the vessels of the superficial papillary plexus of the dermal papilla. There are usually other small infiltrations about small vessels in the upper part of the corium with which it is continuous. The nature of the cells becomes disputable as they acquire bacilli, but if no bacilli are present, they are chiefly lymphocytes and wandering cells such as normally constitute the adventitial cells of the capillaries.] Thus in its simplest form there is nothing characteristic about the cellular infiltration (Dubois). Rare bacilli are found most often in the phagocytic cells about the papillary plexus (Lie, 1935). In ordinary sections the bacilli lie in the cytoplasm, and Klingmüller illustrated minute vacuolar halos about the single organisms. Lie, searching for rare bacilli, was able to find them in all of 10 cases; he stressed the necessity of searching through many sections, or during brief periods of activity. Klingmüller (1927) attributed an origin from the blood stream to tuberculoid lesions and in 1930 made this statement: "The cutaneous macular eruptions of neural leprosy are to be construed as seedings of bacilli from the blood stream." There is little else in the literature that agrees with this strict interpretation of the origin of macules, while references to a hematogenous origin of nodules are abundant.

The central parts of macules have been frequently examined, and Hansen (1882) observed that in most there appears some atrophy of the epidermis, of its hairs and glandular appendages, while the cellular reaction may amount to almost nothing, perivascular lymphocytes, monocytes, tissue mast cells and others. Small nerves may show myelin wholly or partly absent, endoneurial connective tissue increased and Schwann cells increased, i. e., wallerian degeneration or some fibrosis; or it may be impossible to recognize small nerves as such. Laaft found any remaining bacilli broken up and arrectores pilorum muscles atrophic.]

*Nodules.*—Histologically, the variety of macules ranges from Arning's leprid with its rare bacilli to other lesions whose superficial infiltrating cells, though few, are stuffed with prodigious numbers. Since Philippon (1893) wrote of the vascular lesions of leprosy, there have been innumerable suggestions that the bacilli which lead to the formation of nodules reach the skin by way of the blood stream. In rare cases small nodules are sprinkled over the whole body (Soulage and Nadessin).

Herxheimer and also Riecke described accumulations of bacilli in endothelial cells of small vessels, their illustrations being reproduced in Klingmüller's opus magnum of 1930. I have presented reasons for believing that these are rather common developments in old lesions and that they are not a result of embolism of small vessels as Herxheimer

suggested. Probably the early nodules would be found in relatively normal skin, where bacilli have frequently been described. Klingmüller found them there rather commonly, particularly in advanced leprosy, suggesting that of the many that may be deposited few develop into nodules. The development follows the geographic plan of the skin, as infiltrations along or within its structures, until the enlarging infiltrations destroy the intervening connective tissue and coalesce. Even in the solid lesion the separate lobules remain fairly distinct. The epidermis is thinned and flattened, and beneath it is a thin uninvolved layer of collagen fibers separating it from the leproma proper. This boundary strip consists of the stretched papillary layer of the corium.

The individual areas also enlarge by the addition of granulomatous cells and by the spread of bacilli between the fibers of the corium, where they follow and are closely applied to blood vessels. Bacilli can be found a short distance from the main areas, lying free in tissue spaces, and in acute phases these extracellular bacilli are sufficiently common to permit tracing their connection to the parent foci. That bacilli lie within the lumens of such capillaries as well as in their adventitial cells is doubtful, but the supposition is supported by Klingmüller, while Berengrün, and Muir, maintain that the spread is by way of lymphatic capillaries. Individual lepromatous masses often become sharply outlined, but no capsule develops.

#### THE LEPRO CELL

The nature of the bacillus-containing cells that form the bulk of the lepromatous lesion has been one of the most discussed topics in leprosy. Herxheimer stated:

. . . The lepra cells have been variously designated, and we see here a gradual development of beliefs wholly parallel to those with regard to the cells of tuberculosis. Neisser thought of connective tissue cells, but regarded his so-called "globi" as stalactites of lymphocytes. Most French authors considered large mononuclear cells as the basic cells. Then plasma cells were dragged in, as by Gurd, while Unna, who generally denied the existence of "lepra cells," believed that plasma cells surrounded bacillary emboli. Other authors derived the lepra cells from fixed tissue cells. One group thought of them as being principally endothelial cells. Numerous authors found in the skin transitions from *fixed* connective tissue cells to the vacuolated lepra cells, as Virchow indicated.

From the writing that has been done on this subject there issue a few clear points. Opinions are often as much a product of academic tradition as of observation. The nature of the lepra cell is not a question peculiar to leprosy. The cells which differentiate into the vacuolated, bacillus-containing and lipoid-containing Virchow lepra cells occur in any granulomatous lesion. It may be said that leprosy offers one example of how the mononuclear cells of labile capacity are altered under special

conditions. There is little agreement among histologists and pathologists about the origin and interrelation of these cells.

Chuma and Gujo reported the result of injecting lithium carmine and india ink directly into the nodules. It was found that the cells which contained bacilli readily phagocytosed the dye particles. This was confirmed by Koike, and in recent years the treatment of leprosy by intravenous injection of several dyes (Gougèrot and Degos) has further shown that leprosy lesions with their abundant phagocytic cells take up many more dye particles than the normal skin. The cells, especially the multinuclear cells, may occasionally phagocytose various particles, such as hemosiderin (Ermakova) and elastica bodies (Mitsuda).

The numerous theories of the origin of the bacillus-containing lepra cell have been recited and reviewed by Herxheimer, Klingmüller and many others and need not be repeated at length. It is a problem of general pathology, not of leprosy, of which MacCallum wrote:

. . . The attempts directed toward subdivision and classification have not been very successful and efforts to trace their origin have been even less satisfactory. It seems natural to some in any difficulty to say simply that endothelial cells proliferate and produce these cells. But although this unsupported statement has been made the basis of many detailed studies of various infectious diseases and experimental studies, there is not the least actual evidence that it is true.

In the early lesions of leprosy it is not possible to name each and every infiltrating cell. In some rapidly progressing leprosy granulations many of the cells are simply large phagocytic cells such as occur in many diseases except that some have engulfed bacilli and become vacuolated while others are elongated or spindle shaped and not distinguishable from fibroblasts except that they lack the fibrils.

Mitotic figures were seen in lepromas by Virchow and by Malassez, who regarded leprosy as a sarcomatous process. Schmidt went to one extreme: "Neoplastic cells may be derived from the glandular, epithelial, endothelial or even fat cells." Philippon, and Havelberg, also saw mitoses, and subsequent observations have varied greatly. When present, mitoses occur particularly at the margins of the lesions in cells of several kinds. Cowdry, Heimburger and Williams, studying lepromas by the Feulgen reaction, noted the absence of thymonucleic acid, and by microincineration they observed variation in mineral contents, particularly a relatively low amount of calcium ash. Reticulin is elaborated throughout the leproma, while the elastic fibers of the corium are destroyed. Milasch reported the formation of irregular balls of newly formed elastic fibers at the margins of nodules, like the hyperplastic elastic tissue in some cases of atrophic dermatitis.

Philippon pointed out that both the lipoids and the bacilli in some cells occur not in the vacuoles but in the intervening cytoplasm. On the

other hand, it has been a routine observation that even small groups of bacilli in young cells occur within vacuoles, although the lipoids are strictly cytoplasmic. Not all vacuoles are occupied by bacilli. Plasma cells, scattered in loosely arranged foci at random through the nodules, are usually accompanied by some lymphocytes.

#### GLOBI

The masses of bacilli within the tissues are called globi (originally meant by Neisser to designate the vacuoles about the organisms). Cowdry cited Manson-Bahr to the effect that the sizes and numbers of globi present are a factor of the age of the lesion.

In smaller globi the bacilli form conglomerations or bundles arranged in fascicles, the "cigar packs." The shape becomes spherical after enlarging through various irregular forms, a common one being an irregular ovoid form, which Cowdry called a seed globus. The organisms become so closely matted as to appear agglutinated, with individuals often indistinct, while one or more round hollows develop in the centers of some of the larger globi. In old or regressing lesions, degenerating forms are seen to the point of disappearance of bacilli. In fresh smears the globi often fill the vacuole completely, but in sections they are shrunken. Often the vacuole exceeds in size the mass of bacilli, to the point where numerous but scattered bacilli should questionably be called globi.

The presence of a homogeneous material among the bacilli, which stains with several nuclear dyes, was known to all the early students of leprosy. Because the material exhibits also the staining qualities of mucin, it has been called a slimy substance, a conclusion without other substantiation. The botanical term "zoogloea," or "gloea" for short, used by Hansen, suggests a living matrix of the bacilli, an idea equally unfounded. The amount present is closely related to the number of bacilli forming the globus, and large globi exhibit large masses of the material, whereas when the bacilli are not so numerous as to be solidly matted, there is usually none. In old lesions from which bacilli have partly or completely disappeared, this vacuolar material may remain behind, well preserved.

The fluid in vacuoles without bacilli or with bacilli in less than global masses does not react to any known dye. It is not fat, at least not in early stages, and Virchow's statement that the cells apparently take up water is as much explanation as is justified.

The thin delicate lining of the larger vacuoles which separates the vacuole from the cellular elements adjacent is also readily stained by a variety of dyes in no characteristic manner. Berengrün supposed it to be the margin of the endothelium of the lymphatic in which the globus

supposedly lay. It is more prominent with the larger globi, doubtfully existent about small masses of bacilli and absent about simple vacuoles. Those who have argued against the lymphatic idea have found no really adequate explanation of its nature (Denney). Cowdry regarded the membranes about giant globi as remains of investing giant cells.

The larger globi often are not spherical but assume complicated elliptic or ovoid forms, with bulges between adjacent tissue elements, and these in particular have been the source of Unna's claim that globi lie in lymphatics. Cowdry (1940) was particularly interested in what he called giant globi. He acknowledged that all stages of intracellular development from cigar packs to seed globi could be seen. The giant globi occur only in advanced lesions, as a later development. Lie (1894) stated: "Das Charakteristische eines Globus kann nicht die Grösse sein" (Size cannot be a characteristic feature of a globus).

That the smaller masses of bacilli are intracellular can be demonstrated; but the largest, which exceed 100 microns in greatest dimension, often have been assumed to lie free in the tissues. Unna believed that plasma cells surrounded the free-lying bacilli. The large masses often seem to extend or flow a short distance along the tissue spaces, perhaps to have broken out of their original confinements. Multinuclear giant cells are commonly found in relation to the larger globi, as recognized by Rikli, Schäffer, Gurd and others. They often consist of a thin layer of cytoplasm with flattened nuclei, completely surrounding the globus, or they resemble foreign body giant cells. Less frequently they may have peripherally arranged nuclei with more abundant cytoplasm, and in these there may be a few cytoplasmic bacilli in addition to the enclosed globus. It is unusual for giant cells of any size to appear in nodules except in relation to moderate-sized or large globi. Both this fact and the appearance of the giant cells suggest that many are formed only after the globus has reached a considerable size and extended beyond the confines of the cell in which it originated. In occasional lesions or unusual cases, as seen by Ramón y Cajal, Rikli, and Boinet and Borrel, giant cells occur in unusual numbers as a striking feature of the lesions, while their occasional presence has been repeatedly observed by Michelazzi, Dohi, Lombardo, and others. Small multinuclear cells, with two or three or four nuclei, can be found on search in almost any case. Most latter day observers concur in the opinion that these are formed by the fusion of single cells, and Cowdry expressed the opinion that even cells with many bacilli might be included. A rare form of massing of the bacilli radially to form rosettes, described by Wolbach (Gurd), is found much more commonly in rat leprosy (Cowdry).

*Controversy Over Lymphatic Harborage of Globi.*—A feature of the history of the anatomy of leprosy has been the situation created by the



dermatologist Paul Gerson Unna. He described a method of preparing sections stained for bacilli, which included drying the section completely. From these he concluded that the large masses of bacilli lay in lymphatic spaces or ducts and that in fact the bacilli never occurred in cells but only in terminal *Lymphspalten* (lymph clefts). The cellular response was interpreted as secondary to the growth of the organisms in the lymph spaces; the cells plastered themselves against the organisms and their zoogloea. Unna made no exceptions; in all the organs the bacilli were extracellular, lying on and not in the endothelium of blood vessels or of hepatic sinusoids and restricted in distribution to lymph and blood channels. He was answered by Neisser, Hansen and Touton. Neisser was plainly outspoken in his criticism of Unna, calling his methods useless, his material inadequate and his conclusions precipitate. Unna responded without hesitancy in maintaining his views, answering one of Neisser's objections with words which Neisser had addressed to Kaposi at a meeting in Copenhagen: "The important thing is not the number of cases, but how they are studied." He stated the absolute with total finality, "Die Bacillen liegen in der That niemals in den Gewebszellen" (The bacilli, as a matter of fact, never once occur in tissue cells), and, "Since the new tinctorial era of histopathology there has been too much free diagnosis of 'cells'; leprous histology is testimony of this."

Unna had a number of followers, Leloir, Kühne and a dozen others and particularly Berengrün; none seems to have accepted the totality of Unna's "never." Von Bergmann in 1897 remarked that the controversy appeared to have reached a certain laying aside of weapons (*Waffenstillstand*), with acceptance of most of Berengrün's ideas. Furthermore, at this time Jeanselme (1898) said that Unna described himself as a hardened sinner, still maintaining the position.

The importance of the Unna controversy is that it has colored nearly every subsequent histologic investigation of leprosy in some degree. Unna was in gross error on two points. He misinterpreted the Virchow lepra cell; looking for nuclei within globi, he failed to find them and mistakenly thought that the globi were what Virchow had described as cells. His absolute denial of the presence of bacilli in cells is accepted by nobody; not even Unna's most enthusiastic pupils followed him to this extreme, yet Unna himself, having taken his stand, adhered to it steadfastly, unswayed by argument or demonstration, and without embarrassment. The followers of the lymphatic school adopt an oversimplification of the histology of leprosy as though saying, "The lymphatics tell the whole story."

In 1885 any crevice between tissue elements was referred to by some as a lymph space. But with several in Unna's school any cells enclosing bacilli automatically became lymphatic endothelium. There is no report

in the literature of the injection of the lymphatics of a leprous lesion. The literature on leprosy from the standpoint of pathology is filled with compromising statements where the subject of lymphatics enters, and uncritical acknowledgments of the importance of these structures continually appear. For instance, Muir and others (1923) wrote a long article on leprosy from the standpoint of pathology in which they talked much of lymphatics but conspicuously never mentioned lepra cells. In MacCallum's textbook one reads that bacilli "occur chiefly in the swollen endothelial cells of the lymphatics and blood vessels," embodying a sentiment denied elsewhere. The ghost of Unna has yet to be laid.

Of the relationship of the globi to lymphatics Cowdry (1938) wrote:

Reconstruction of some of these globi from serial sections show one or more delicate channels leading off the lumen through the side of the globus possessed of the thinnest wall. I have not been able to trace them very far, neither have I been able to find them connected with all globi. The openings are narrow, of the same caliber as the channel and not funnel-shaped. They are rather like lymphatic capillaries.

Perhaps even this was compromising with Unna, for later (1940) he wrote:

. . . It is not feasible to trace continuity between the investment of a giant globus and the walls of a true lymphatic.

Carter described lesions in lymph channels, as did Hoggan. Doutrelepon is one of those who leaned toward Unna, seeing bacilli in lymphatics to the point of incredibility. Sakurane derived lepra cells from lymphatic endothelium, and Dwijkoff wrote expansively on the role of the lymphatic system. Among the champions of the Unna doctrine, Berengrün (1895) wrote, "The bacillus-thrombus is the primary thing; it acts as a foreign body stimulating the endothelium of the vessel." This is a little different from Unna's idea that the cells were plasma cells (a cell of more than one genus to Unna).

Some leprous lesions will show dilated lymphatics at the margins of foci, which are identifiable with reasonable certainty. Cowdry spoke of having seen bacilli in the endothelium of such vessels, and I have seen them in this situation twice. It is altogether probable that some of the older writers have seen similar lesions, but with the lymphaticists the descriptions have to be well salted before taking. It has been said many times that in the peripheral sinuses of the lymph nodes bacilli never occur in the flat lining endothelium, a pertinent, if negative, sort of evidence (Cowdry) that there is no great tendency of true lymphatic endothelium to become infected.

The observation that infiltrating leprous foci occur along or around cutaneous blood vessels and nerves has led to the statement that this

spread is by way of the lymphatics, which occur richly in these sites. However great the possibility that this is the case, the lesions overgrow and spread beyond the lymphatic wall promptly, and demonstration of the lymphatics themselves is usually impossible. Aleixu described 13 cases of leprosy with acute lymphangitis.

Von Bergmann remarked that it was apparent from every bacillus-rich preparation that bacilli lay free in the tissues. Klingmüller spoke of extracellular branching chains of bacilli covered by endothelium, but Gurd found these extracellular masses to be merely in tissue spaces. Such chains of bacilli differ from the globi masses which Schäffer, like Cowdry, was able to follow through several serial sections. But the only ones that I have been able to illustrate were extending between fat cells, unrelated to endothelial or other cells and connected with masses of bacilli in nearby cellular infiltrations.

Leprosy of the lymph nodes is histologically like that of the skin, with the formation of vacuolated cells and globi. The regional nodes are regularly involved, but the visceral nodes seem to escape except when they drain a leprosy liver or other involved organ. The bacilli and cells are found earliest in the cortex but may increase to replace the whole node, without spreading into the tissues beyond. Unna Jr. and Lowe (1939) described tuberculoid changes in lymph nodes.

#### ACUTE REACTIONS; ALLERGY

No formal studies of the acute phases of leprosy from the standpoint of histology appeared until lately (Stein, 1939; Büngeler and others; Ermakova, 1940; Rabello Jr.). Stein studied lesions twelve hours, one day and two, three and four days after the beginning of the reaction. Even at twelve hours he found collections of lepra cells in deeper layers containing lipoid and many bacilli, and perivascular cells developed in the course of a few days into typical lepromas. Fibrinoid swelling of connective tissue fibers, which were yellow with Van Gieson's stain, was seen after twenty-four hours, with various acute changes in small vessels, hemorrhages and leukocytic extravasations. He attributed this to a hyperergic inflammation akin to the Shwartzman phenomenon but recognized that many of the apparently new lesions were old ones made evident by the acute reaction. Ermakova described a fatal acute reaction with hemorrhagic changes in the lesions, degeneration, and want of acid-fastness of the bacilli in reactive nodules, and I have seen a similar picture with widespread acute necrosis of all the infiltrations and with colonies of non-acid-fast organisms, apparently lepra bacilli, in the lymph nodes. In general, in acute phases the bacilli occur in extremely numerous but small separate intracellular bundles and, in sections, lack individual distinctness (Fernandez). Büngeler and colleagues observed the circulatory phenomena in connection with the acute reaction, edema

and fibrin being present in the tissues in early stages, and likewise considered this evidence of an allergic state. Later eosinophils and leukocytes appeared with decreasing numbers of bacilli, and a third healing phase was described. They have also divided acute reactions into types, according to the types of lesions. Traditionally, the acute or lepra reactions are accompanied by the appearance of new lesions and the activation of old ones which may have lain dormant months or years. They vary from the rare profound systemic reactions, which lead to death, to the mild ones, in which the histologic changes consist of, nothing more than the suggestion of rapidly advancing lesions. The changes in the acute reactions of tuberculoid leprosy, described by Wade, Büngeler and a few others, are largely of this sort, perhaps with the presence of bacilli previously not demonstrable, although some of the circulatory phenomena have been described in connection with them.

The necrosis taking place in leprous lesions in relation to acute reactions is not uniform and does not appear to be a regular feature. The ulcerations which so commonly occur on the crests of acutely inflamed nodules doubtless arise from small superficial areas of necrosis. In deeper nodules suppuration with fluctuancy but without ulceration is sometimes apparent, and I have seen a few instances in which this necrotic material was *not* laden with bacilli and in which small ulcers occurred on the surface of old, almost obsolete nodules without any increase in the few, poorly staining organisms present. In old long-staining lesions in which the tissues, if not the patient, are approaching mortification, secondary infections may lead to broad putrid sloughs, which in the extremities will sometimes correspond with areas of total anesthesia. On the "trophic ulcer" of leprosy, that *horrendus malus morbus*, the histologic literature has been kind by saying little. Histologically, it is like the bed sore, a first cousin. *Erythema nodosum* (Chala) is another phenomenon not too uncommon in leprosy, which may have an allergic significance, together with some bullous or pemphigoid lesions.

*Pigment Changes.*—"Leprosy," said Gilbertus Anglicus in the thirteenth century, "is an infection or alteration of the natural color of the skin to an abnormal or uneven color with the equality of flesh." The mediaeval German poet, Conrad von Würzburg (via Babes and Virchow) wrote of a leper:

Des Leibes Farbe, sonst zu schauen  
In früherer Zeit so licht und gut,  
Sie war viel röther noch den Blut,  
Und gab so sonderbaren Schein.

(The color of the body, which  
In earlier days had been so clear and normal,  
Was now much redder than that of blood  
And gave a most peculiar appearance.)

While Holcomb shows that Gilbert and other mediaeval writers mixed leprosy in with syphilis and other diseases, the color changes in leprosy may be as dramatic as they describe. The most common is the reddening of the lesions which occurs with acute reactions, while some macules exhibit from the first nothing but depigmented patches. In some of the more florid lesions there is a period beginning with the height of activity during which marked hyperpigmentation occurs over the active area.

In the depigmented patches Henderson noted no change in the melaniferous apparatus other than that the pigment was lowered in amount in its normal sites, as shown by silver stains. Muir (1923) saw little but increases or decreases in amount comparable with the clinical evidences. Wade, Stein and many others have agreed that the pigmentary changes are secondarily brought about, either through local stimulation during acute phases or through atrophic changes, associated with neural disturbances.

#### LEPROUS CHANGES IN SKIN

*Sweat Glands.*—Since 1860 it has been known that infiltrations of the sweat glands are a prominent occurrence in cutaneous lesions of all kinds, the larger infiltrations separating the coils and often preserving them embedded in solid lepromatous tissues. The involved glands do not respond to pilocarpine as do normal glands, with a profuse production of sweat.

Hoggan's description of the changes in sweat glands is confused by his erroneous ideas of the nature of leprosy. Touton produced a beautiful illustration of a sweat gland with numerous bacilli in the gland cells and lying free in the ducts. Michelazzi expressed the belief that such bacilli were thereby eliminated from the body. Spillman and others found them here rather commonly; Ishizu, rarely. Des Essarts and Lefrou (1937) described the various infiltrating cells in detail, as well as various degenerative changes in the glands proper, and expressed the opinion that bacilli passed through unbroken glands into the sweat. Although it is argued by others that this is an important basis of the spreading of leprosy, infection of the gland cells is not a routine occurrence.

*Sebaceous Glands.*—The involvement of the sebaceous glands is of much the same order with bacilli rarely if ever found therein, as described by Cornil and Babes, and Ishizu, while degenerative, pressure and atrophic changes are the rule in advanced lesions, which also affect the hair follicles. In some tuberculoid lesions I have seen the sebaceous glands more frequently the centers of granulomatous foci than the sweat glands.

*Epidermis and Hair Follicles.*—Cornil and Babes, Touton, Thoma, and many others since, observed bacilli in the epithelial cells of hair

follicles. Only a few, e. g., Guttmann, have failed to see them here. Bacilli within cutaneous epithelial cells may be observed especially among cells near the base of the hair follicle and in the epidermis near ulcerated margins of involved areas or elsewhere related to regenerating epithelium. At the root of the hair, the cells surrounding the papilla are often involved, while the papilla itself with its rich vascularity may be diffusely leprous. The epithelial cells infected are sometimes those of the bulb at the root forming a new hair, or those of the external sheath of the root nearby. It commonly happens that all or a group of hair follicles in a particular area be similarly affected, although other lesions, equally heavily laden with bacilli, show no invasion of epithelial cells. Bacilli may be found also along the hair shaft, but it frequently happens that the hair has been shed, and the attempt to regenerate a new hair has been unsuccessful. Degenerative or atrophic changes in hair follicles without bacillary invasion are more common.

The epithelial cells containing bacilli show some vacuolation but do not accumulate lipid, and bacilli, although numerous, do not form in masses as globi. Muir and Chatterji (1932) have suggested that bacilli escape to the surface through the interstices between the epidermal cells, and Klingmüller noted that typical lepra cells were not formed from the epithelial cells. He and various others have shown that bacilli may be demonstrated in epithelial cells scraped from the surface of many nodules. The importance of the "follicular apparatus," stressed by Muir (1936) and Stein (1940) as affording a starting point for leprous foci, depends on the vascularity of the papilla of the hair follicle, not on infection of epithelium.

*Lipoids.*—The earliest stain for lipid to be used was osmic acid, reported by Hansen (1871) and shown to be capable of staining the bacilli. Iwanowsky's fatty changes seem to have been inclusions of neutral fat from fat tissues in nodules. Philippon (1893), using osmium, described finely granular lipid in the walls of vacuoles or in the cytoplasm of the bacillus-containing cells, showing that a small amount of cytoplasm stretched between the vacuoles was thus demonstrated by osmium when not apparent otherwise (Storch). Mitsuda showed that sudan III stained the lipoids well and the bacilli slightly. MacCallum (1916) observed the presence of much fat in leprous nodes, and found it not doubly refractile. Salvioli confirmed this, but Cedercreuz found both neutral and doubly refractile fat in 2 cases. This has not been confirmed by Herxheimer or by many others who have since agreed on its absence.

Mitsuda employed the Fischler, Ciaccio and Smith methods as well as Nile blue and Sudan, as did Herxheimer, finding the lipid stained in some manner by all; Sudan gave it not the bright red of neutral fat but a

brownish color. He concluded that it was lipid of indefinite composition containing fatty acid, while Herxheimer said that the Smith-Dietrich method showed it to be a mixture of esters of cholesterol, glycerin and fatty acids containing some free fatty acid.

The lipid appears in the bacillus-containing cells as soon as the bacilli. At first it is strictly in the cytoplasm of the cells, the vacuoles being quite free, but it increases with the age of the lesion, gradually compressing the vacuoles; old lesions may be so filled as to give the tissue a greasy texture and then the tissue stains brilliantly with sudan in the gross.

*Verrucous Lesions.*—De Souza Araujo (1937) reported 3 cases in which he felt the verrucous lesions might be due to an added fungus-infection, which he was unable to demonstrate. Braga contributed 2 more examples of this. The changes in the skin, marked acanthosis with edema and nonspecific chronic focal inflammation in the dermal papillae, correspond to the disease which American textbooks designate as dermatitis vegetans. I have seen a dramatic example of this, with warty lesions covering most of the lower parts of the legs, marked by much putrid bacterial surface growth over the unbroken but anesthetic skin.

#### LESIONS OF BLOOD VESSELS

Although Uhlenhuth found bacilli in the intima of the aorta and in that of the jugular vein, the vessels involved are principally those associated with lepromatous lesions of the skin (and nasal passages and testes). In the skin, vessels of any size may be infected in one way or another, and virtually any cell, endothelial, smooth muscle or connective tissue, may show bacilli.

Bacilli in endothelium were shown by Cornil and Babes, and Touton described the organisms in intima, media and adventitia. Rikli, Gurd, Schäffer and many others have shown bacilli in capillary endothelium, and when present there they are usually observed in endothelial cells of both arterioles and the venules supplying them (Fite); such invasions are extensive in some areas, but absent from others.

The endothelial cells containing organisms become swollen and even vacuolated, and although fair numbers of bacilli develop, there is no lipid, and the organisms do not form solid globi as in the lepra cells. Philippon found the bacilli in capillaries parallel to the long axis of the vessel, lying near the nuclei, but the statement is correct when only few bacilli are present. Infected cells may extend as a complete inner sheath into moderate-sized veins (Sakurane).

Perivascular leprous foci are so common that the dividing line between adventitia and leprous infiltration is obscure, and although it is proper to speak of a general infiltration of the adventitia, intimal or endothelial

involvement is rare compared with this (Glück, 1898). Philippson noted that the bacilli in the endothelium stain better, and Henderson's illustration of this, as well as those of Riecke, Herxheimer and Klingmüller, indicates that many of the intimal and endothelial lesions are comparable to those of the epithelium: They occur in older nodules as a secondary invasive phenomenon.

Infiltration of larger vessels appears to take place via the vasa vasorum, but smooth muscle fibers containing bacilli have been seen only in small arterial branches. Large veins of the extremities rarely show leprous thrombophlebitis (Philippson, 1899), although the general absence of thrombi even from most heavily infected blood vessels shows how little cellular injury the lepra bacillus provokes. Doutrelepon and Wolters found bacilli in the clots in large vessels. Rivelloni made capillaroscopic studies of the skin, and Ota and Sato found obliterative endarteritis in tuberculoid lesions.

#### LESIONS OF NERVES

The involvement of the peripheral nerves is generally considered an ascending infection from the skin. Little is known concerning the involvement of the sensory nerve endings with the exception of Pacini's and Meissner's corpuscles. Hoggan before the lepra bacillus was known described obliteration of the pacinian bodies, believing this to be the result of nerve atrophy. Sudakewitsch later gave an extensive description of the bacillary invasion of the pacinian corpuscles, observing that the bacilli lay between the lamellated plates and along the vessels; when bacilli were numerous, there were granulation cells filling a central cavity. The central nerve fiber was always atrophied. Older processes showed atrophy and fibrosis of the corpuscle with disappearance of the central nerve fiber. As to Meissner's corpuscles, Dacco noted their destruction in a case of anesthetic leprosy. Saijo and Takino showed that many normal corpuscles were to be found, while the greatest damage resulted where bacilli were most abundant; they also found degeneration of nerve endings in muscle fibers. Later Takino and Miyake showed masses of bacilli arising in Pacini's and Meissner's corpuscles and in taste buds. Askanazy proposed that leprosy primarily involves the terminal nerves, whence lesions are neurolepomatous in origin. Torssujew studied silver impregnations of the fine terminal nerve fibers in which he saw breaks and nodular thickenings, some of which apparently represented attempts at regeneration. MacCrae noted "the separation of the two sensations of touch and pain by leprosy." In late years the differentiation of sensations of heat and cold from those of pain or touch effected by the anesthesia of leprosy has received much attention, but the anatomic basis of these peculiarities is obscure.



DeBeurmann, Gougerot and Laroche wrote of a patient with extensive leprosy of four years' duration but with cutaneous sensation intact, and Leloir (1886) said "on a certainment trop exagéré cette constance de l'anesthésie" (certainly this constancy of anesthesia has been much exaggerated). I should be inclined to dismiss these observations except for the experience of an autopsy in a case in which there was extensive bacillary infiltration of all the cutaneous nerves of the face, where the infection was heaviest, and sensation was unaltered. For this, perfect preservation of nerve ending seems necessary though many nerve fibers were destroyed.

Except for the apparatus of Rezzonico, the Schmidt-Lantermann incisura and the nodes of Ranvier, bacilli have been demonstrated in every part of the nerve fiber. It is generally accepted (Ermakova) that the numbers of bacilli present correspond roughly to the numbers in the cutaneous lesions and that the nerves associated with nodules may be as rich in bacilli as the lepromatous infiltrations themselves. Virchow saw lepra cells enclosing individual nerve fibers. According to Cornil, Babes was the first to demonstrate bacilli in leprous nerves in 1881.

The leprous lesions of the small branches in the skin are the same as those of the main nerves. In the average more or less active lepromatous lesions the nerves are found infiltrated by bacilli and cells, with degeneration of myelin sheaths and axis-cylinders, together with proliferation of the connective tissue of the perineurium and the endoneurium. The picture as a whole is complex, and the thick ulnar nerves described by Danielssen, Virchow, Arnott, Arning, Langhans, Arning and Nonne, and others are not only the result of old lesions but the product of both progressive and regressive changes taking place simultaneously.

That organisms occur inside the myelin sheath, i. e., in the axis-cylinder, has been specifically stated by Lie (1894), Uhlenhuth and Takino. Others have employed the statement "bacilli occur in nerve fibers" (Wynne; Guttmann; Arning; Ermakova) but have not specified the axis-cylinder or the myelin sheath. Although Lie stated that stains for the myelin sheath will also stain lepra bacilli, this is not universally true, especially if paraffin rather than celloidin<sup>2</sup> sections are used. I have seen a case of acute neuritis in which the the central canals normally containing axis-cylinders showed numerous bacilli, often in solid masses distending them and smoothly enclosed by myelin. Although Sokolowsky presented the opposite view, that bacilli never occur in nerve fibers, and Kellogg in teased preparations found the organism only on the surface of nerve fibers and not within, it is possible that actual growth along axis-cylinders is more common than has been proved. Higher up in the main nerves it is often possible to show single bacilli in axis-cylinders.

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2. Celloidin is a concentrated preparation of pyroxylin.

Dejerine and Leloir used osmium to show degeneration of myelin in 1881, as did Hansen, Babes and some others shortly thereafter. A few years later, with the introduction of Weigert's method, degeneration was shown by that method. Actively leprous nerves usually show every degree of demyelination in one or another group of fibers. In small branches in the skin the myelin is often completely gone, while the bacilli appear to be increasing in numbers. Although there are many statements that bacilli may be found more readily in terminal nerve branches than elsewhere in the skin in mild leprosy, I have seen only a rare instance in which this has been so.

Bacilli have been described in the myelin sheath by Takino, with globi arising there. Nonmyelinated nerves usually are found uninvolved, although Takino, and Ermakova, showed the usual leprous changes in the sympathetic vertebral chains and ganglions in cases of the nodular form with extensive involvement of the nerves. Takino also found bacilli in the vagus nerve, an isolated observation.

The presence of bacilli in Schwann cells has been claimed by Sokolowsky, Uhlenhuth and a host of others and denied by Cowdry (1940). Multiplication of the Schwann cells as seen by Shaw is a common finding, attributable to wallerian degeneration. The identification of Schwann cells in some leprous nerves is a doubtful possibility, particularly when other mononuclear cells have infiltrated the involved nerves widely.

There is marked hyperplasia of the fibrous cells of the perineurium, and although an increase of endoneurial connective tissue cells is not so marked, a diffuse increase in endoneurial collagen is extensive (Woit). Bacilli have been found in the connective tissue cells from early days (Hoggan; Babes, 1897; Arning, 1884; Rikli; Wynne), usually singly or as small compact bundles without vacuolation, lying close to the nuclei. The perineurium becomes a thick collar of the nerve, quite cellular at first, but contracting about fibrous nerve bundles, in which a few normal fibers may persist.

There are always some infiltrating cells—lymphocytes, plasma cells, mast cells and mononuclear cells. The last may contain bacilli, with development of globi. In the early stage of their development into lepra cells they often present the appearance of simple phagocytes, and they and other infiltrating cells are found particularly about blood vessels. The organization of these cells to form solid leprous nodules is infrequent, although Grieco (1938) said that in cases of the nodular form of leprosy the lesions of nerves result particularly from bacillus-laden vacuolated cells producing compression of nerve fibers. Mitsuda and Ogawa described lepromatous changes in "certain" cases, and Lowe spoke of having seen them, as I have, rarely. But for the most part the cells infiltrate the nerve along vascular pathways and along clefts between nerve fibers.

Dejerine and Leloir expressed the belief that the cutaneous lesions might represent spread from the nerves, but Delio (1889) and Gerlach proposed a now classic diagram of the spread from the skin to the nerves and from one nerve to another where branches join. Gerlach emphasized the possibility of metastatic nerve lesions, also favored by Mora Guarnido; Muir and Chatterji (1936) suggested that bacilli might pass up the nerve via the cutaneous neurovascular plexus, without producing lesions of the skin, while they, and Takino also, proposed that spread along the nerves was by way of the lymphatics of the nerves, an idea flatly contradicted by Maximow's, ". . . these vessels have not been found . . . in peripheral nerve trunks." Raynaud's bodies and other similar degenerative lesions (Arning and Nonne) occurring above the sites of activity in old cases are not directly or characteristically related to leprosy, if at all. The perineural spaces which are sometimes illustrated by carcinomatous infiltrations about nerves are obliterated by the proliferation of connective tissue in leprosy.

*Abscesses of Nerves.*—Combemale and Marestang described caseous cavities inside leprous nerves, distending the nerves to various degrees, with bacilli not demonstrable in some but common in others (Marestang). Arning (1899) described an early abscess of a nerve. Lie (1905) mentioned occasional abscess formation with calcium deposits in the ulnar nerve at the elbow, and Muir (1924) saw a typical lesion, while Lowe (1929) contributed 19 examples, with others being added by Wade (1934), Lowe (1934), Ota and Sato (1934), Schujman, Chatterji, Bosq, Grieco and Nolasco. These have consisted of elongated caseous masses within the perineurium, particularly that of the ulnar nerve above the elbow, sometimes rupturing through the nerve casing. Histologically, they show large amounts of central caseous material surrounded by a zone of typical tuberculoid granulomatous tissue with large epithelioid cells and giant cells. Bacilli are usually absent and rarely common. Schujman suggested that an allergic state contributes to their formation. Calcification appears to follow the caseation as it does in tubercles.

*Central Nervous System.*—The older literature of leprosy deals extensively with a possible relation between leprosy and syringomyelia, or Morvan's disease (Steudener; Langhans; Pestana and Bettencourt; Voit). Lie (1905) dismissed it completely on the basis of his own studies. Many others who studied the central nervous system in leprosy failed to find changes akin to those of syringomyelia.

A second confusing finding of former days was degeneration in the posterior columns of the spinal cord in clearcut cases of leprosy. Arnott expressed the opinion that it might be of a postmortem nature, but later writers attributed the degeneration to leprosy. The possibility of

syphilis as a cause of such degeneration was given no thought by Colella and Stanziale, Looft, Jeanselme and Marie, or Voit. Lie (1905) further described such lesions, but this was the critical date ne plus ultra, and in 1930 he abandoned the former position. Others, e. g., Storch and Babes (1897), found no changes in the spinal cord, and Vilde recently concluded that the only lesions of the spinal cord are those secondary to arteriosclerosis, while Ermakova (1936) found the central nervous system wholly normal.

The occurrence of bacilli in the spinal, gasserian and other ganglions in cases in which there was heavy infection of the nerves is well established (Sudakewitsch; Lie; Uhlenhuth; Babes; Natali). Rarely bacilli have been seen in neurons of the anterior horn of the spinal cord (Andriani), in Purkinje cells (Uhlenhuth) and in the pia (Doutrelepoint and Wolters). There is the curious isolated case of de Beurmann and others of leprous meningitis and pleuritis.

#### LESIONS OF THE RESPIRATORY TRACT

*Nose and Throat.*—Leloir (1885) described leprosy of the nose and pharynx as being usually a broad superficial infiltration, and Glück (1897) indicated that in many cases the lesion remains flat and infiltrates the deeper tissues only to a moderate extent. Elevated or projecting nodules may also be found but less frequently than the flat lesions and only in cases of well advanced nodular leprosy. Hollmann found the nose infected in 89 per cent of cases of nodular leprosy, 66 per cent of cases of mixed and 45 per cent of cases of anesthetic leprosy, and many similar compilations have given comparable results. In the earlier cases Akamatzu showed that the apparently intact nasal mucosa may contain numerous leprous infiltrations crowded with bacilli.

Wade and others have shown that a common early site of leprosy of the nose is the cartilaginous-bony juncture of the septum, the cartilaginous septum often being destroyed with resulting perforation. Glück saw infiltration of the perichondrium and of the cartilage cells with bacilli, though the latter was "not an everyday experience." Both he and Akamatzu found vascular lesions rather common and involvement of nerves the rule.

Further involvement of the upper air passages may become most extensive or even diffuse. The epiglottis is easily destroyed, and von Bergmann noted that an area of efflorescence of the mucosa ulcerated much more readily than one of the skin. Laryngeal lesions destroy the vocal cords, and resulting fibrosis or granulomatous masses may necessitate tracheotomy (Breda, 1908). In the lower areas normally covered by columnar epithelium, squamous metaplasia of the epithelium attempting to recover ulcerated areas is common (Lie). The mucous and

serous glands become infected, with bacilli in secretory cells and ducts, and metaplasia of the epithelium of these ducts follows regularly. Extension into main salivary glands is, however, rare. Of the 5 heavy infections of the larynx that I have seen, all were in persons dead of advanced pulmonary tuberculosis, and the laryngitis was a mixed tuberculous and leprosy process with both diseases flourishing and intermingling.

*Lung.*—In the older literature there is much confusion between tuberculosis of the lung and so-called leprosy of the lung (e. g., the articles by Bonome, Délépine and Slater, Riehl, Babes and Moscuna, and Arning [1898]). Fambri reported a case he believed to be one of pure leprosy of the lung, yet his case, Bonome's and the case reported by Babes and Moscuna were almost surely simply instances of tuberculosis with plentiful bacilli. Hansen and others had doubted the occurrence of such a disease as pulmonary leprosy, and Doutrelepon found only tuberculosis. Scagliosi argued against pulmonary leprosy, and Schäffer considered it most rare. The case of de Beurmann and co-workers is not free from suspicion.

Jeanselme (1911) recognized minute leprosy foci in septal walls, some within capillaries, but doubted that any other change occurred in the lung in leprosy. Sugai reported something similar, having found occasional bacilli in otherwise normal lungs. Mitsuda (1936) stated:

. . . The bacillus is to be seen in the histiocytes in the interstitial tissue, within endothelial and perithelial cells of the blood vessels, and in the dust cells. There are also groups of lepra cells that are sometimes from 0.01 to 0.1 mm. in diameter. The dust cells, heavily laden with coal pigment, change into the vacuolated cells upon the entry of the bacillus and stain orange by Sudan III.

Kobayashi saw similar lesions, and Tajiri described rare minute septal and subpleural foci, particularly as demonstrated by fat stains. In the majority of cases reported, no lesions at all have been found.

#### LESIONS OF OTHER ORGANS

*Liver.*—Leprosy of the liver is transparently the result of vascular seeding. Rake (1892) wrote that he "had never seen any visceral macroscopic changes which could with certainty be ascribed to leprosy." Sabrazès said, "Properly speaking, leprosy nodules are not found in the liver." Müller and Mertodidjojo found gross lesions always to be tuberculous and not leprosy.

Kupffer cells containing bacilli are a constant finding in the liver in cases of nodular leprosy (Hansen; Neisser; Guttmann; Babes). Mitsuda pointed out that bacilli in Kupffer cells are often well preserved in form and tinctorial properties while those in other hepatic foci are fragmented and beaded, the difference being due to a difference in ages.

The Kupffer cells readily become vacuolated and, according to Bertelotti, tend to develop into lepra cells but do not complete the process. They may contain large masses of bacilli, as in the case of Sabrazès, or only a few. In some cases it appears from the widespread diffusion of small numbers of organisms in single cells, and the infrequency of larger foci that few of the bacilli phagocytosed by the Kupffer cells from the blood stream reproduce further. Larger foci may develop from the Kupffer cells to become slightly elongated and distend the sinus, a delicate fibrous framework developing with reticulum and capillaries.

Similar foci, often called miliary lepromas, develop commonly in two other positions, the portal areas (Dehio, 1876) and along the walls of emissary veins. It is usual to find these at autopsy with rather few bacilli and a good deal of lipoid, often obsolete. Their limited individual size also speaks for a brief period of activity and ready regression.

Salvioli, Andriani, Sugai and de Beurmann have attributed cirrhotic states to leprosy. However, there is no good reason to suppose that these are other than Laennec's cirrhosis occurring independently in patients with leprosy. The presence of bacilli in hepatic cells has been recorded by Andriani, Leloir, Rikli and Jeanselme, but Mitsuda (1936) stated my own experience: "Rods that simulate leprosy bacilli are often found in liver cells, but they are nothing but fat or bile pigment crystals."

*Spleen.*—The lesions of the spleen are comparable to those of the liver, being similar in size, or smaller, and of the same character. Neisser (1886) noted that they were particularly found about blood vessels. The foci are chiefly like those in the Kupffer cells, occurring where small vessels or capillaries open into the pulp, in or at the margins of malpighian bodies and along small trabecular vessels. In light involvement, as in the case reported by Dwijkoff, there may be little more than scattered vacuolated cells with a few bacilli, which it would be impossible to demonstrate without staining for acid-fast bacilli or fat. Although in extreme involvement innumerable foci are found occupying a good part of the tissue, heavy bacillary infection, as in the case of Sabrazès, is uncommon. Tuberculous lesions are so often seen in leprous livers and spleens that minute leprous foci may develop in the outer epithelioid cells of unquestionable tubercles.

There is a much larger literature on leprosy of the liver and spleen than I have indicated. Biehler wrote a monograph on leprosy of the spleen, and Schäffer reviewed visceral leprosy in 1898, as did Jeanselme in 1900. Mostly volume has been added to the subject since, except that Pineda called attention to some cases of cutaneous leprosy apparently without bacilli in which persistent search revealed a few organisms in nerves and viscera.

*Testis.*—Whereas leprosy damages the liver and the spleen to no important degree, it frequently destroys the testis as a functioning organ (Schäffer). Cornil and Babes saw large numbers of bacilli in the testis, and Leloir observed that the testis was almost always involved soon or late in nodular leprosy. Neisser (1881), Storch and Rikli saw rich bacillary infection of the testis between the tubules only, but it has been demonstrated that the organisms may proliferate in the tubules, in the spermiogenic cells (Kinoshita; Kobayashi) and perhaps in the Sertoli cells. Hansen (1893) found the bacilli in the tubules, both within and outside the cells, producing huge irregular masses that almost filled the tubular canals.

Thoma showed that the interstitial infiltrates consisted of the usual various cells, lymphocytes and predominantly those which develop into typical Virchow cells. They lead eventually to a high degree of sclerosis (Natali), with atrophic tubules remaining as islets in dense scar tissue, or there is diffuse fibrosis between hyaline tubules, with sometimes scattered islands of Leydig cells. The blood vessels and nerves may be affected, but in the testis proper the process rarely becomes lepromatous with the formation of nodules. It is usually a diffuse interstitial infiltration intense in degree but thin in volume, limited by the tunics of the organ.

Bacilli may occur in the epithelium lining the ducts of the rete, rarely in that of the first part of the epididymis, although the neural infiltration extends well into the epididymis and occasionally small lepromatous nodules are formed along the pampiniform plexus.

*Bones.*—There are two common changes in the bones in leprosy. One of these is the presence of scattered minute cellular and bacillary foci in the red marrow, which are comparable to those seen in the liver and the spleen (Babes). Gass and Rishi found the marrow infected in 17 of 20 cases of nodular leprosy and free from bacilli in 48 cases of neural involvement. The other common change is the absorption of the terminal bones of the extremities, particularly the phalanges. Study of these atrophic changes by roentgenograms (Harbitz; Dubreuilh; Honeij) shows that the bones undergo a peculiar type of atrophy and resorption, quite independent of nodular activity (Hirschberg and Biehler), in which whole phalanges waste and vanish. The marrow becomes adipose (Haüpl), the bones become very soft from loss of calcium, and resorption by osteoclasts follows leisurely or is not conspicuous. The normal trabeculation collapses with little change in the basic structure. These resorptions occur without infection of the bones by lepra bacilli or by secondary infecting organisms, although purulent osteomyelitis is a common complication.

The relation of these alterations of bone to the anesthasias accompanying them has led to their designation as trophic changes. Yet in nearly all writings there appears dissatisfaction with this as the complete explanation, "These bone changes seen so early and which are so gradual, particularly in the nodular type of leprosy are not . . . sufficiently accounted for" (Honeij).

Actual leprous infiltrations of bones, rare enough to be a most unusual experience, have been described by Hallopeau and Lebret, Rath de Souza, Mitsuda and Ogawa (in the form of cranial periostoses), Hirschberg and Biehler, and Sawtschenko. The last in his case saw bacilli in osteoclasts, in haversian canals and endothelial cells and in the periosteum.

*Eye.*—The lesions of the eye have attracted much attention since Bull and Hansen published their book describing punctate keratitis, spreading conjunctival nodules and some of the secondary changes. Direct extension of leprous lesions of the skin to the conjunctiva and thence to the cornea is an important mechanism by which the eye is involved. As described by Babes and Levaditi, the bacilli pass from the limbus to the cornea, later penetrating the ciliary body and the iris, with ulceration and perforation of the cornea possible. The lens is not invaded but may be absorbed. The bacilli pass from the ciliary body along the ciliary nerves to the equator of the eye, but the posterior part of the eye is not invaded. Franke and Delbanco described the changes seen in a case of leprosy of the milder type in which bacilli occurred at the angle of the anterior chamber, particularly about blood vessels in the iris and the ciliary body, but suggested that the eye might become involved by the hematogenous route (Borthen and Lie), with the occurrence of numerous minute granulomas scattered throughout the uvea.

The corneal involvement, keratitis punctata superficialis leprosa, as studied by Philippon, Borthen, Masuda, Breda (1913) and others depends on the spread of bacilli between the connective tissue plates of the cornea. The bacilli are found mostly within but also outside cells, globus formation being rare, even though the organisms are fairly numerous. Masuda saw the organisms in spindle cells under Bowman's membrane or between it and the epithelium. The cornea apparently remains avascular in spite of the leprous infiltrations.

Secondary lesions of the eye of wide variety, such as the chorio-retinitis described by Bistris, may follow the leprous process, yet there is no tendency of the infection to spread into the retina or the optic nerve. Although Lie found bacilli in the optic nerve, it seems clear that this invasion is not comparable to that in the peripheral



branches of the somatic nerves. The lesions of the uvea may become granulomatous like those of the skin.

*Gastrointestinal Tract.*—Von Reissner described leprous lesions of the large intestines in 3 cases, but to his report and other reports of this nature by Danielssen and Boeck, Arning and Monastirski there attaches suspicion, especially since von Reissner did not think there was too much distinction between leprosy and tuberculosis histologically.

*Kidney.*—Bacilli rarely lodged in glomerular capillaries were observed by Rake, and the observation was confirmed by Nonne, Wynne, Brutzer, Sokolowsky, Sugai and Kobayashi. Here they seem scarcely to produce lesions.

*Ovary.*—Glück and Wodynski reported 6 cases of ovarian involvement with interstitial lepra cells containing bacilli and also much brown pigment. Gentili saw the same. I have seen a single ovary with a few leprous foci, and in view of numerous negative findings the process here must be considered unusual.

*Mammary Gland.*—Powell claimed that of 302 males with leprosy 79 per cent had enlarged nipples; he considered the enlargement of the nipples an important diagnostic point. Muir (1934), Pinnetti and Sapo Baretto described enlargement of the male breast (gynecomastia) associated with or dependent on atrophy of the testes. Tissi contributed more material, and Baptista (1937) reviewed the literature, giving new examples.

Babes found bacilli in the female mammary gland. Sugai found bacilli in the milk of 2 nursing mothers. One of these was found to have bacilli only in smooth muscle cells of the nipple, at the poles of their nuclei; the other, in lepromatous infiltrations of the capsular connective tissue of the gland.

*Placenta.*—Sugai found bacilli in the placenta and in the blood of the heart of a newborn fetus, but the most interesting finding is that of Pineda, of whose 104 placentas 55 showed bacilli on smear.

*Thyroid, Parathyroid, Pineal, Adrenal and Pituitary Glands.*—The adrenal gland not infrequently shows small miliary leprous foci in cases of heavy infection, like those of the liver, occurring near the vessels or at the margins of the cortex and the medulla. In the other glands of internal secretion Pinnetti and Natali showed in several cases complex nonleprous changes, probably associated with testicular atrophy. Muneuchi's analysis of 45 parathyroid and 10 pineal glands showed foamy cells (not necessarily with bacilli) in 37 of the parathyroid and all the pineal glands.

## ANOMALOUS OBSERVATIONS

There are inevitable curiosities which turn up in so multiplex a disease as leprosy. Askanazy likened leprosy to von Recklinghausen's disease, and Johannsen and McCreary found numerous bacilli with globus formation in the neurofibromatous neoplastic cells of the lesions of a patient with both diseases. Black saw bacilli in cancer cells in 2 lepers. Ciaccio reviewed the subject of keloids in leprosy. Pautrier wrote of a case in which there were multiple cutaneous hematomas filled with organisms. Lara's theory of ptomaines and leprosy belongs with Jonathan Hutchinson's doctrine of fish-eating and Webb's curse of vaccination as spreading the disease.

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## Notes and News

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**Appointments, Deaths, Etc.**—David J. Davis retires next September 1 as professor and head of the department of pathology, bacteriology and public health, and as dean, of the University of Illinois College of Medicine. He became professor of pathology in 1914 and dean in 1925.

Martin H. Knutsen, professor of bacteriology in Pennsylvania State College, died February 6, 55 years old.

Eugene Markley Landis, professor of internal medicine in the University of Virginia, has been appointed professor of physiology in the Harvard Medical School, Boston, succeeding Walter B. Cannon, retired.

David L. Coffin, of the department of veterinary pathology of the University of Pennsylvania; has been appointed Herbert Fox memorial fellow in comparative pathology by the Zoological Society of Philadelphia.

**Institute of Medical Research.**—The Institute of Medical Research of the Toledo Hospital, Toledo, Ohio, was dedicated March 27. The institute, established and maintained by a legacy of the late Frank Collins, will be under the direction of Bernhard Steinberg, the director of the laboratories of the Toledo Hospital.

**Society News.**—The 1943 officers of the Society of American Bacteriologists are Rebecca C. Lancefield, president; I. L. Baldwin, vice president; W. B. Sarles, secretary-treasurer.

The American Public Health Association will sponsor a three day Wartime Public Health Conference in New York city, October 12, 13 and 14 in connection with the seventy-second annual business meeting of the association. The conference will be devoted exclusively to wartime emergency problems of public health.



## Book Reviews

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**Autonomic Regulations: Their Significance for Physiology, Psychology and Neuropsychiatry.** Ernst Gellhorn, M.D., Ph.D., professor of physiology, University of Illinois College of Medicine. Pp. xii and 373, with 80 illustrations. Price \$5.50. New York: Interscience Publishers, 1943.

Throughout all of Professor Gellhorn's writings there is an undercurrent of philosophy that is at one time highly illuminating and at others obscure. The present volume begins with a quotation from Sherrington stating that physiology "aims at giving reasoned accounts of the acts of an organism in respect of their purpose and use to the organism *qua* organism. This may be called a teleological aim, yet belongs to a teleology not foreign to the scope of natural science." Professor Gellhorn seeks in the present volume to present "an organismically oriented" system of thinking, and one of his chapters is entitled "Contributions to an Organismic Physiology." This implies that "every adaptation is an integration," and Professor Gellhorn intimates that the reactions of the body to anoxia, asphyxia, hypoglycemia and other conditions which involve the autonomic nervous system illustrate the essential unity of the body for the reactions in question and tend to restore the body to the original condition. Thus the autonomic nervous system is presented as the principal agent of bodily homeostasis. From the philosophic standpoint the volume will be stimulating since, among other things, the author has grasped the importance of the hypothalamus as a primary center of autonomic integration, and he lays emphasis on the fact that all phases of bodily metabolism are ultimately regulated not by the endocrine system in the first instance but by the nervous system.

Although every physiologist is interested in a philosophic unification of the organism and will therefore welcome Professor Gellhorn's presentation, it is not quite clear for whom the book is primarily intended. Medical students will find it valuable as collateral reading, but it will not be an essential textbook since it lacks morphologic detail. It would similarly be helpful for one interested in the surgery of the autonomic system, though scarcely adequate, since there is little reference to practical application and almost none to recent surgical developments relating to the autonomic system. Indeed, it is curious that a book with such an admirable bibliography (1,100 references with full titles) omits reference to the most valuable recent monograph on the autonomic system, namely, the second edition of "The Autonomic Nervous System," by J. C. White and Reginald Smithwick, published in 1941. The subtitle of Gellhorn's book suggests that it may be useful to psychologists and neuropsychiatrists. The chapters on the autonomic basis of emotion can indeed be warmly recommended, as can that on the autonomic system and neuropsychiatry.

One of the principal contributions of Professor Gellhorn and his school has been in the study of the effect of anoxia on the various divisions of the autonomic system, but these papers have nowhere been summarized, and it is a pity that there are only scattered references in the present volume to this timely and important work. Similarly, although the author is eager to establish the primacy of the nervous system in the control of the endocrine glands, details concerning the innervation of these glands, especially the innervation of the pituitary, on which much of his argument hinges, are largely omitted.

Among the virtues of the volume under review is the excellent folding frontispiece prepared by W. R. Ingram, of the University of Iowa, giving a diagram of the hypothalamus and its intracortical connections. A second and conspicuous virtue of the book is that each chapter is systematically summarized at the end. Finally, the bibliography, already mentioned, is not only highly useful but a model of accurate, careful preparation, albeit White and Smithwick are omitted.

**Ovarian Tumors.** Samuel H. Geist, attending gynecologist, Mount Sinai Hospital; clinical professor of gynecology, College of Physicians and Surgeons, Columbia University. Pp. 527 with 312 illustrations. Price \$10.50. New York: Paul B. Hoeber, Inc., Medical Department of Harper & Brothers, 1942.

This book deals with ovarian tumors in the light of the recent advances in the embryology, anatomy and physiology of the ovary. These advances are reviewed in the first three chapters. Then come chapters on general considerations of ovarian tumors; on "benign" epithelial neoplasms of surface origin; proliferating cysts; carcinoma of the ovary; primary parenchymatous epithelial neoplasms of subsurface origin (granulosa cell tumor, theca cell tumor, arrhenoma, dysgerminoma, Brenner tumor); ovular tumors (dermoid, teratoma); stromatogenous (mesoblastic) ovarian tumors; follicular cysts, lutein cysts, endometrial cysts, hematoma, granulomatous lesions, etc.; ovarian tumors in children; par-ovarian tumors; ovarian tumors in pregnancy; diagnosis and treatment of ovarian tumors. This list not only describes the general contents of the book but illustrates the author's histogenic classification of ovarian tumors. Here "stroma" signifies the musculofibrous framework with vessels and nerves and "parenchyma" the epithelial mass in the body of the ovary, that is to say, the follicular apparatus, the granulosa and theca internal cells, and the embryonal remains in the medulla and rete. The classification as well as the text would have been simplified and improved if "cancer" and "cancerous" had been used in place of "malignant neoplasm" and other such terms, and "malignant." And would it not be preferable to use "choriocarcinoma" or "chorioma" in place of "chorio-epithelioma"? At the end of each chapter is an elaborate bibliography of, as a rule, several pages and covering mainly gynecologic writings in the English and German languages. The book is copiously, not to say overabundantly, provided with photographic illustrations of gross and microscopic appearances, practically all original. The magnification and the staining of the microscopic figures are not given except that in a few cases special methods are mentioned. On page 238 "cell inclusion" is used not to mean, as it usually does, cytoplasmic or nuclear inclusion but inclusion of cells, e. g., adrenal cells within ovarian tissue. And "secondary carcinoma" (page 243) does not mean, as commonly understood, metastatic carcinoma but carcinoma developing in a previously noncarcinomatous tumor. The description of the structure and manifestations of ovarian tumors is excellent. The style is methodical and easily understood, though there is a tendency to use more words and to give more details than necessary. Instructions as to treatment are given in broad outlines as each tumor is described. In the last two chapters the diagnosis and the treatment of ovarian tumors in general are discussed. The chapter on treatment deals mainly with surgical procedures. The experience with radiotherapy does not lead to any definite conclusions. No instructions are given as to methods: "It is essential to utilize adequate radiotherapeutic measures if one is to expect good results," but one is not told what are adequate radiotherapeutic measures. All in all, this is a highly useful, up-to-date, competent book on ovarian tumors.

## Books Received

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ATLAS OF OVARIAN TUMORS. Gemma Barzilai, M.D., New York. Preface by Fred W. Stewart, M.D., pathologist, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. Pp. 264, 8½ by 11, with 258 original illustrations, 45 in colors, on 58 plates. Price \$10. New York: Grune & Stratton, 1943.

REPORT OF THE SECRETARY OF THE SMITHSONIAN INSTITUTION AND FINANCIAL REPORT OF THE EXECUTIVE COMMITTEE OF THE BOARD OF REGENTS FOR THE YEAR ENDED JUNE 30, 1942. Pp. 112. Price 25 cents. Washington, D. C.: United States Government Printing Office, 1942.

MEDICAL JURISPRUDENCE AND TOXICOLOGY. John Glaister, M.D., D.Sc., fellow of the Royal Faculty of Physicians and Surgeons, Glasgow: Barrister-at-Law of the Inner Temple; regius professor of forensic medicine, University of Glasgow; formerly professor of forensic medicine, University of Egypt, Cairo, and medico-legal consultant to the Egyptian Government. Seventh edition. Pp. 679 with 132 illustrations. Price \$8. Baltimore: The Williams & Wilkins Company, 1942,

DISEASES OF THE BREAST, DIAGNOSIS, PATHOLOGY, TREATMENT. Charles F. Geschickter, M.A., M.D., lieutenant commander, Medical Corps, United States Naval Reserve; director of the Francis P. Garvan Cancer Research Laboratory; pathologist, St. Agnes Hospital, Baltimore. With a special section on treatment in collaboration with Murray M. Copeland, M.D., F.A.C.S., instructor in surgery, Johns Hopkins Medical School; visiting surgeon and assistant oncologist, University Hospital, University of Maryland Medical School; visiting oncologist, Baltimore City Hospitals. Pp. 829 with 593 illustrations. Price: \$10. Philadelphia: J. B. Lippincott Company, 1943.

## CORTICAL NECROSIS OF THE ADRENAL GLANDS ASSOCIATED WITH ADDISON'S DISEASE

REPORT OF EIGHT CASES

JOHN D. DUFFIN, M.D.

TORONTO, CANADA

Addison's disease in the majority of cases is due to tuberculous destruction of the adrenal glands. In Addison's series of 11 cases<sup>1</sup> there was 1 in which "the two supra-renal capsules . . . appeared exceedingly small and atrophied." The nontuberculous nature of the disease in such cases was later established, and the condition of the adrenal glands has subsequently been described under many names, of which the most popular in the English literature has been "adrenal atrophy." Until recently the frequency of "adrenal atrophy" as a cause of Addison's disease has been generally estimated to be about 10 per cent on this continent and somewhat higher in Europe.

In the department of pathology of the University of Toronto interest in this subject was recently stimulated by the study of 3 cases. A survey of the autopsy records of the Toronto General Hospital from the beginning of 1924 until the time of writing revealed 17 cases of Addison's disease in a series of some 7,000 autopsies on adults. In 10 of these the condition was due to tuberculosis, and in 7 (41 per cent) to "atrophy," of the adrenal glands. Although this series is not large, the figures are in agreement with those of several reports published during the past decade,<sup>2</sup> which indicate an incidence of "atrophy" as a cause of Addison's disease much higher than 10 per cent.

Relatively few cases of Addison's disease associated with the type of lesion under consideration have been recorded in detail. For this reason and in an effort to demonstrate that the process is one of cortical

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1. Addison, T.: *On the Constitutional and Local Effects of Disease of the Supra-Renal Capsules*, London, S. Highley, 1855; *M. Classics* 2:239, 1937.

2. (a) Millis, G. C., and Conybeare, J. J.: *Guy's Hosp. Rep.* 74:369, 1924. (b) Susman, W.: *J. Path. & Bact.* 33:749, 1930. (c) Barnard, W. G.: *ibid.* 33:765, 1930. (d) Duff, G. L., and Bernstein, C.: *Bull. Johns Hopkins Hosp.* 52:67, 1933.

necrosis rather than atrophy, the following 8 cases are added to those already recorded in the literature. The autopsy findings are given in some detail; only brief summaries of the histories are presented.

#### CASE 1

*History.*—A 44 year old white woman, unmarried, was well until the spring of 1922, when she suffered a "bilious attack" which lasted for a week. A short time afterward a yellowish brown discoloration of the face and body was noted. This did not involve the scleras, and persisted until death. A second "bilious attack" of one week's duration occurred a few months later. In the interval and until the final attack the patient felt "fairly well" and continued with her work as a dress designer. The final illness, which began one week before death, was ushered in by general weakness and lassitude. This was followed by nausea, severe vomiting and dehydration. The systolic blood pressure was 110 mm. of mercury at the beginning of the last illness but fell considerably during the three days before death. Before leaving for the hospital the patient superintended the packing of her bag but by the time she was carried downstairs she was comatose, and death occurred on the way to the hospital, Feb. 23, 1924. No clinical diagnosis was made.

*Autopsy* (one hour after death).—The autopsy was restricted to an examination of the abdomen. The skin had a slightly wrinkled appearance, and there was a diffuse brownish pigmentation of the face and to a lesser extent of the body. The subcutaneous fat was reduced in amount. The abdominal organs with the exception of the adrenal glands showed no gross lesions.

Microscopically, the kidneys contained dense focal collections of closely packed lymphocytes just beneath the capsule and in the interstitial tissue of the medulla. The malpighian bodies of the spleen were unusually large and numerous. The gastric mucosa showed many large lymph follicles, some of which had well developed germinal centers. Several small lymph nodes from the adrenal region showed dilated sinuses filled with reticulum cells. No other lesions were found.

*Adrenal Glands.*—On gross examination no adrenal tissue was found on either side. Blocks were taken from the fatty tissues of both adrenal regions for microscopic study.

Microscopically, there was no trace of adrenal tissue, the only structures encountered being normal ganglions and blood vessels, enclosed by fat and areolar tissue.

#### CASE 2

*History.*—A 39 year old white man was admitted to the Toronto General Hospital July 1, 1926 and died July 5, 1926. The patient, a butcher, was well until 1922, when he had "influenza." Subsequent to this he suffered from periodic attacks of weakness, coming about once in three months and lasting usually for three days. One month before admission he noticed severe weakness and "gas on the stomach" and began to vomit after meals. He became progressively weaker so that he was unable to sit up in bed. On admission he appeared ill and dehydrated and showed definite bronzing of the face and the hands. The pulse was feeble and thready, and the blood pressure was 50 systolic and 34 diastolic. The red blood cell count was 7,100,000 and the hemoglobin content 94 per cent. A diagnosis of Addison's disease with addisonian crisis was made. He failed to improve and died quietly in his sleep.

*Autopsy* (twelve hours after death).—The body was well developed and moderately well nourished and showed patchy brownish pigmentation of the face, the hands and portions of the body. The heart, which weighed 220 Gm., was

extremely flabby, and there was a dark red blotchy subendocardial hemorrhage over the anterior wall of the left ventricle. The gastric mucosa contained many petéchiial hemorrhages. The spleen weighed 150 Gm., and the malpighian bodies were unusually large. The thymus was not identified, and the lymph nodes showed no enlargement or other abnormality. The thyroid gland, the pituitary gland, the brain and the testes were not examined. The rest of the organs with the exception of the adrenal glands were essentially normal.

Microscopically, the tissues failed to show any additional lesions.

*Adrenal Glands.*—Because of their very small size, the adrenal glands were located only after a rather prolonged search. The left gland was represented by a small mass of tissue of the approximate shape of an adrenal gland but so thin as to be almost translucent. It was quite firm in consistency, and the cut surface was of a uniform grayish color and failed to show the normal markings. The right gland was even smaller than the left but closely resembled the latter in other respects.

Microscopically, the two glands presented a strikingly similar picture. Over large areas no cortical tissue was present, only a thin strand of medullary tissue and collapsed cortical stroma intervening between the two layers of the capsule. The remaining cortical tissue took the form of irregular masses of cortical cells, infiltrated with lymphocytes and occasional large mononuclear cells and separated by bands of collapsed stroma and newly formed connective tissue. These masses of cortical cells varied in size from 200 to 700 microns and showed no semblance of normal cortical structure and nothing to suggest a zonal arrangement. The individual cells without exception were degenerate in appearance and irregular in size and shape. The majority were considerably larger than normal with indistinct outlines, granular and sometimes vacuolated cytoplasm, and nuclei which were either pyknotic, misshapen, fragmented or absent. Many of the cortical cells were frankly necrotic, and a moderate amount of cellular debris was present (fig. 14). The vascular channels of the cortex were prominent and engorged with red blood cells. In some areas there were small amounts of blood pigment lying within phagocytic mononuclear cells, but this was not a striking feature. The medulla was small in amount, densely infiltrated with lymphocytes and showed a moderate increase in fibrous tissue. The small pericapsular arteries were thick walled, and although the capsule itself was normal, in several situations collapsed cortical stroma lying immediately beneath it gave an erroneous impression of capsular thickening.

### CASE 3

*History.*—A 31 year old white woman was admitted to the Toronto General Hospital Sept. 24, 1926 and died Oct. 2, 1926. Her previous illnesses included influenza, in 1918, and several abortions. She had had no full term pregnancies. Two years before death she began to complain of weakness and anorexia, which necessitated her staying in bed about one week in four. The "weak spells" persisted and on occasion were associated with nausea and vomiting. In the intervals she was "tired all the time." Nine months before death she first noticed bronzing of the skin, which increased steadily in depth for about a month, until she was quite brown. The final attack of weakness and vomiting began five weeks before death. On admission to the hospital she was poorly nourished, restless and dehydrated. There was brownish pigmentation of the skin, the lips and the buccal mucosa. The pulse was weak and poorly maintained, and the blood pressure was 78 systolic and 58 diastolic. The red blood cell count was 5,200,000 and the hemoglobin content 65 per cent. The Wassermann test of the blood was very strongly positive. No history of treatment for syphilis was obtained.

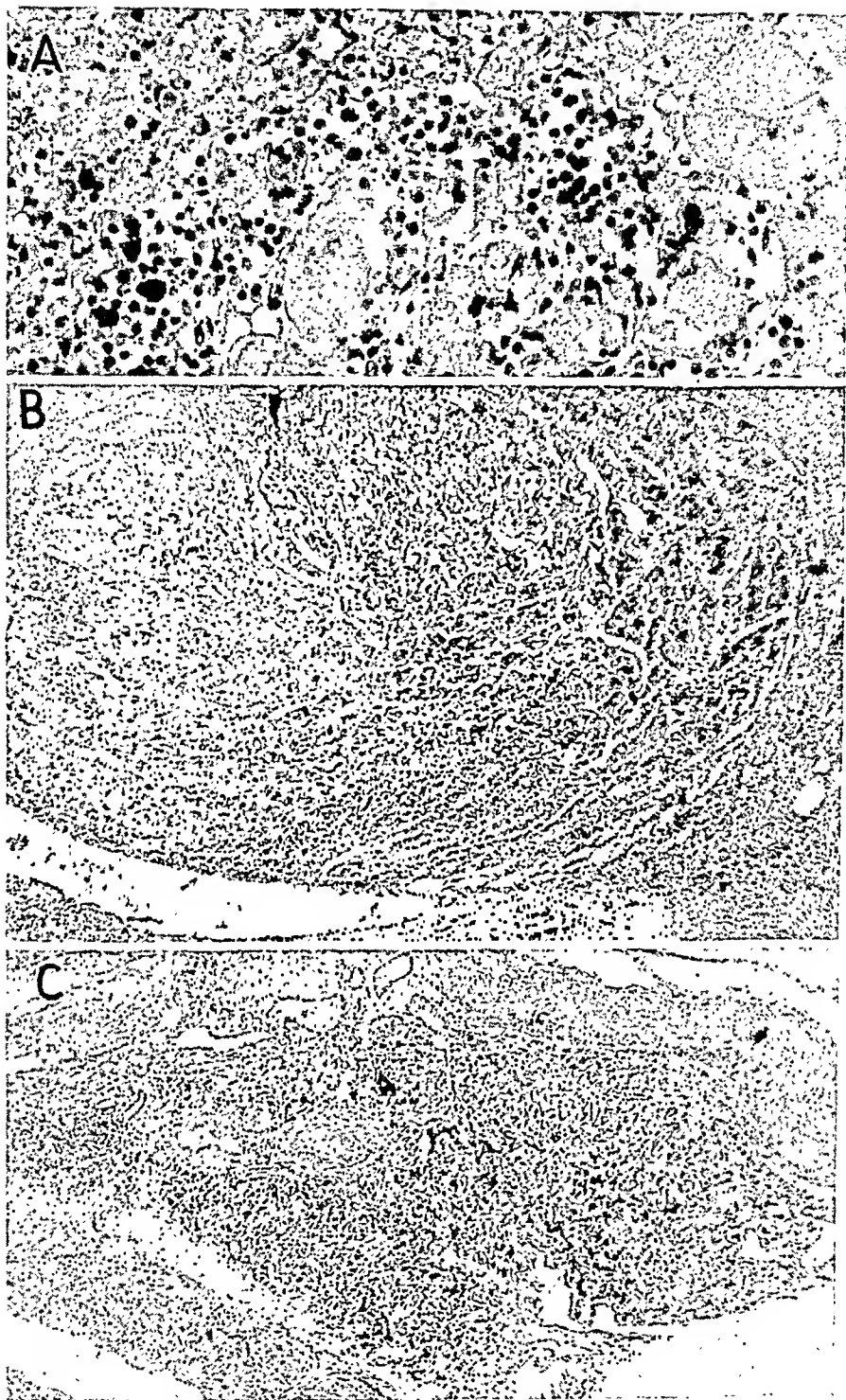


Fig. 1.—*A*, portion of one of the irregular masses of degenerate and necrotic cortical cells in case 2. Hematoxylin and eosin;  $\times 250$ . *B*, largest of the cortical nodules in case 3. It measured 2.5 mm. in diameter. Hematoxylin and eosin;  $\times 40$ . *C*, section from remaining adrenal tissue in case 4. A normal amount of medullary tissue lies between the thickened capsule (above) and the central vein (lower left). No cortical tissue is present. Hematoxylin and eosin;  $\times 40$ .

A diagnosis of Addison's disease and syphilis was made. The vomiting continued, and the patient failed rapidly; the blood pressure dropped to 58 systolic and 38 diastolic three days after admission and was unobtainable for two days before death occurred.

*Autopsy* (eight hours after death).—The body was greatly emaciated, and all the bony landmarks stood out prominently. There was uniform dark pigmentation of the skin, together with patchy pigmentation of the gums and the soft palate. The thymus was readily identified, although its weight was not recorded. The heart weighed 205 Gm. and the spleen 105 Gm. These were normal in appearance, as were the pituitary gland, the thyroid gland and the ovaries. The fallopian tubes were bound down to the posterior pelvic wall by old adhesions. The subcutaneous and abdominal fat tissue was of a peculiar deep yellow color, verging on orange. The other organs with the exception of the adrenal glands showed no lesions.

Microscopically, the pituitary gland showed much diffuse fibrosis of the anterior lobe with a paucity of cells and a great scarcity of basophils. The normal ratio between acidophils and chromophobes did not appear to be altered. The thyroid gland showed moderate fibrous tissue replacement of glandular elements and marked lymphoid hyperplasia, frequent lymph follicles with well developed germinal centers being present. The acini, which were of normal size, were lined by low cuboidal epithelium and filled with homogeneous colloid. The thymus gland resembled that of an infant in that it was made up of closely packed lymphocytes without fatty replacement. The leptomeninges and the mucosa of the bladder showed focal collections as well as a diffuse infiltration of lymphocytes, while the kidneys also contained small interstitial collections of similar cells.

*Adrenal Glands.*—Despite a careful search of the retroperitoneal tissues the adrenal glands were not found; however, in microscopic sections of tissue removed from the adrenal regions the glands were identified.

Microscopically, these glands bore a close resemblance one to the other. The larger portion of each gland was composed of compact fibrous tissue infiltrated with lymphocytes, which were distributed both diffusely and in focal collections. Except for two small nodules on each side, no cortical tissue remained. The largest of these nodules (fig. 1 B) measured 2.5 mm. in the greatest diameter; the other three were considerably smaller but closely resembled the former in other respects. The normal cortical structure was entirely absent, each nodule being made up of jumbled masses of irregularly arranged cells, the whole apparently representing an island of cortical tissue which had escaped complete destruction. Most of the individual cells were well preserved, but many showed irregular cell outlines, coarsely granular cytoplasm and nuclear pyknosis or karyorrhexis. A few showed definite necrosis. These cortical remnants were irregularly infiltrated with lymphocytes but not to the same degree as was the surrounding fibrous tissue. The medulla on each side was moderately abundant and was grouped largely about the central vein. Here, too, there was fairly dense lymphocytic infiltration with increase in mature connective tissue. The medullary cells, although somewhat reduced in number, were of the usual irregularly polygonal shape and of normal appearance. The capsules, which were uniformly thickened, blended almost imperceptibly with the abundant fibrous tissue which made up the major part of the damaged glands.

#### CASE 4

*History.*—A 35 year old white woman, married, was first admitted to the Toronto General Hospital June 18, 1933. She complained of weakness, anorexia and darkening of the skin of one year's duration. The patient was poorly



nourished and showed brownish pigmentation of exposed surfaces and pressure points. The blood pressure was 90 systolic and 60 diastolic. A diagnosis of Addison's disease was made, and the patient was discharged feeling somewhat improved and taking 10 Gm. of sodium chloride daily. In July 1934 she was readmitted, unconscious, in hypoglycemic coma. Consciousness was regained after the intravenous administration of dextrose solution. Subsequent hypoglycemic attacks were controlled at home by the taking of dextrose drinks. During the succeeding fifteen months the patient felt generally better than she had since the onset of the illness. The final admission was on Nov. 25, 1935. For the preceding several weeks she had noticed a slow return of severe weakness and ready exhaustion. Two days before admission severe diarrhea and increasing weakness had developed. Examination in the hospital showed an exhausted but conscious deeply pigmented woman. The blood pressure was 90 systolic and 60 diastolic. The blood sugar amounted to 31 mg. and the blood sodium to 280 mg. per hundred cubic centimeters. Dextrose and saline solutions administered intravenously failed to improve her condition, and death occurred Nov. 27, 1935, following a period of coma.

*Autopsy* (one hour after death).—The body showed moderate emaciation, and there was diffuse light brown pigmentation of the skin, with many dark brown freckles. The pubic and axillary hair was scant. The organs tended to be small, the heart weighing 160 Gm. The retroperitoneal lymph nodes were enlarged, but the other lymph nodes were normal. The kidneys were contracted and had a granular surface, and the ovaries were small and fibrosed. No trace of the thymus gland was present. The thyroid gland was atrophic, weighing only 10 Gm. The pituitary gland was grossly normal, as were the other organs, including the pancreas. The spleen weighed 120 Gm.

Microscopically, the kidneys showed chronic glomerulonephritis with extensive fibrous tissue replacement of parenchymal elements. The thyroid gland showed multiple large foci of lymphocytic hyperplasia and moderate diffuse fibrosis. The anterior lobe of the pituitary gland contained an excessive amount of connective tissue, and there was an almost complete absence of basophil cells, with a reduced number of eosinophils.

*Adrenal Glands*.—No evidence of adrenal tissue was present in the gross specimen.

Microscopically, adrenal tissue was readily recognized in blocks taken from the adrenal region of each side. On the right side only the capsule with the central vein and a very small amount of adjacent adrenal medulla were present. There was complete absence of cortical tissue. The medulla consisted of only a few normal-appearing chromaffin cells clumped around the central vein. The capsule with its irregular foldings outlined the characteristic contour of a diminutive, very thin adrenal gland. This capsule, however, was uniformly thickened, and except in the area of the central vein there intervened between its two layers only a small amount of fatty areolar tissue, containing an occasional lymphocyte and a few thin-walled vascular spaces. The left gland differed from the right only in its greater content of adrenal medullary tissue. No cortical tissue was present. There was a considerable amount of medulla, which encircled and spread out for some distance on each side of the central vein. The actual volume of medullary tissue did not seem to be greatly reduced, although the contained parenchymal cells were considerably diminished in number. There was a diffuse increase in fine connective tissue in the medullary portion of the gland, together with a greater than normal prominence of thin-walled vascular spaces and a diffuse lymphocytic infiltration (fig. 1 C). At each extremity of the left gland large

areas were seen where, as in the right gland and as in cases 3, 5 and 7 (fig. 2 C), only a thin layer of areolar tissue separated the two layers of the uniformly thickened capsule.

#### CASE 5

*History.*—A 35 year old white woman, married, was first admitted to the Toronto General Hospital Dec. 28, 1934. She gave a history of weakness, loss of weight and darkening of the skin and pigmentation of the lips of seven months' duration. The immediate cause of her admission was acute nasopharyngitis. Her past illnesses included septic abortion, in 1928, and "rheumatism," in 1930. Examination showed in addition to the nasopharyngitis typical addisonian pigmentation and a blood pressure of 85 systolic and 60 diastolic. A diagnosis of Addison's disease was made. While she was in the hospital, the Wassermann reaction of the blood was found to be strongly positive. The blood sodium varied in amount from 291 mg. to 322 mg. per hundred cubic centimeters. The lowest value for blood sugar during fasting was 77 mg. The patient was discharged improved on a high intake of sodium chloride. She remained fairly well until January 1936, when she was readmitted in crisis with an infection of the upper respiratory tract. The blood pressure was 74 systolic and 50 diastolic, and the blood sodium amounted to 290 mg. per hundred cubic centimeters. Despite intensive antisiphilitic treatment in the interval, the Wassermann reaction was still strongly positive. She improved rapidly on intravenous injections of saline solution and extract of adrenal cortex. During a three months' stay in the hospital she received frequent injections of the extract and large amounts of salt by mouth. She seemed to be in satisfactory condition on discharge from the hospital but was readmitted acutely ill four days later, April 18. She died within forty-eight hours with signs of bronchopneumonia.

*Autopsy* (two hours after death).—The body, which was well nourished, showed diffuse light brown pigmentation of the skin and a scarcity of pubic and axillary hair. There were bronchopneumonia involving the right lung and old inflammatory disease of the fallopian tubes. The thymus gland was not identified, and the lymph nodes showed no enlargement. The heart and the spleen weighed 235 and 190 Gm., respectively. The pituitary gland and the ovaries were normal in size and appearance; the thyroid gland weighed only 20 Gm. but was normal in configuration and consistency. No other gross lesions were present except in the adrenal glands.

Microscopically, the pituitary gland showed the usual proportion of eosinophils, basophils and chromophobe cells and no fibrosis. The thyroid gland exhibited a marked degree of lymphoid hyperplasia and moderate diffuse fibrosis. None of the other organs showed lymphocytic infiltration.

*Adrenal Glands.*—The left gland weighed 1.1 Gm. and measured 4 by 1.3 by 0.4 cm. The right gland weighed 0.6 Gm. and measured 4 by 2 by 0.2 cm. Except for their difference in thickness, the glands were similar in appearance and of the usual shape. They were yellowish brown, and the cut surface showed a grayish yellow outer layer, which appeared to be the cortex, and a grayish white central portion, which apparently represented the medulla. The cut surface in some areas showed only a grayish white capsule, without either cortex or medulla between its two layers.

Microscopically, the right gland contained a small quantity of cortical tissue and an apparently normal amount of medulla. The latter showed numerous aggregations of lymphocytes and a slight diminution in the number of parenchymal cells with, as in most of the other cases, an increased prominence of thin-walled vascular channels. Otherwise the medulla was normal, the individual polygonal

cells being well preserved and arranged in the usual manner. The cortex was represented by a thin layer, which partially invested the medulla and central vein, and by a small discrete subcapsular nodule, which was completely isolated from the rest of the adrenal tissue by a broad fibrous band (fig. 2*A*). In both situations the cortical tissue was either extremely degenerate or necrotic. None of the usual cortical structure remained, nor was there any trace of a zonal arrangement.

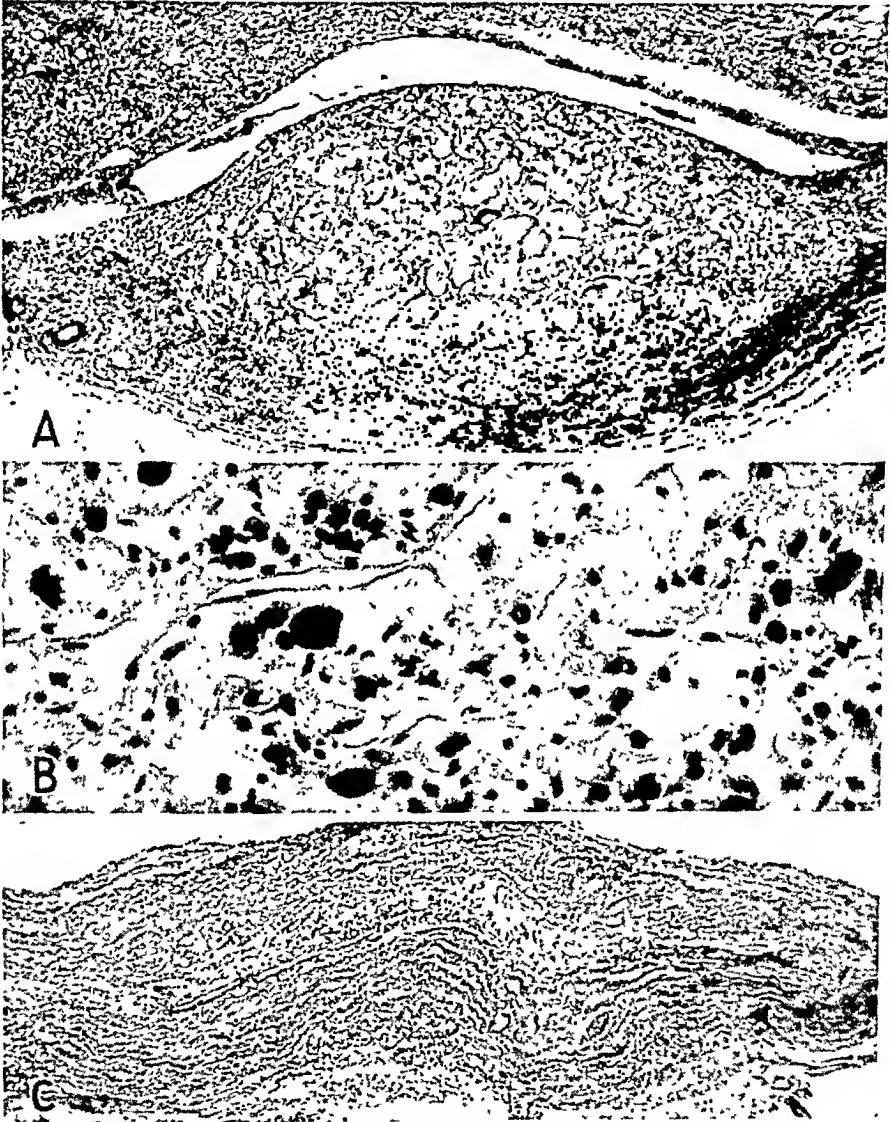


Fig. 2.—*A*, subcapsular cortical nodule in case 5. Hematoxylin and eosin;  $\times 40$ . *B*, high power view of the degenerate cortical tissue in case 5. Hematoxylin and eosin;  $\times 250$ . *C*, two layers of the thickened capsule in case 5 separated by only a thin strand of loose connective tissue. Hematoxylin and eosin;  $\times 40$ .

The cells almost without exception were much larger than normal and ballooned in appearance. In some cases the cell outlines had disappeared and the fused cytoplasm of adjacent cells gave the impression of giant cell formation. The cytoplasm was coarsely granular and in many instances vacuolated, while the nuclei for

the most part were pyknotic, fragmented or absent. A few of them were extremely hyperchromatic and much larger than normal (fig. 2 B). In several situations groups of fat cells were present lying among cortical cells, some of which they had apparently replaced. Toward the periphery of the isolated cortical nodule a diffuse infiltration of lymphocytes was seen, while the rest of the cortical tissue contained varying numbers of these cells, both irregularly scattered and in focal collections. In approximately one third of the gland neither cortical nor medullary tissue remained; here the two layers of the capsule were separated by only a thin strand of loose connective tissue (fig. 2 C). The left adrenal gland resembled the right in all essentials: The cortex was degenerate and greatly reduced in amount; the medulla was relatively well preserved, and the whole contained varying numbers of lymphocytes.

#### CASE 6

*History.*—A 26 year old white woman, married, was first admitted to the Toronto General Hospital May 28, 1939. She gave a history of weakness and ready fatigue of three years' duration and of daily attacks of vomiting for two weeks. She was exhausted in appearance and markedly dehydrated. The skin of the face and the hands was deeply pigmented. The blood pressure was 60 systolic and 40 diastolic. A diagnosis of Addison's disease was made. She improved rapidly following treatment with saline and dextrose solutions and extract of adrenal cortex. She was readmitted to the hospital in crisis twice in 1938 and twice in 1939. One crisis was associated with idiopathic pleurisy, another with menstruation, and still another followed removal of an infected wisdom tooth. In the intervals she felt fairly well. In November 1940 pellets of desoxycorticosterone acetate were implanted in the subcutaneous tissues of the left infrascapular region. The final admission was on Jan. 19, 1941. The patient was admitted in crisis and died a few hours later.

*Autopsy* (two hours after death).—The body, which was normally developed and moderately well nourished, showed brownish pigmentation and dark freckling of the face and the hands. The significant findings exclusive of those in the adrenal glands consisted of fibrinosanguineous pericarditis, enlargement of the thymus glands, which weighed 20 Gm., enlargement of the cervical, abdominal para-aortic and mesenteric lymph nodes and of Peyer's patches of the ileum, and a thyroid gland which weighed only 15 Gm. but was otherwise normal in appearance. The other organs, including the ovaries, presented no abnormality. The heart weighed 240 Gm. and the spleen 150 Gm. The cranial contents were not examined.

Microscopically, the thyroid gland showed lymphocytic infiltration and diffuse fibrosis. The individual acini, although reduced in number, were of normal appearance with a normal colloid content. The thymus gland was composed of densely packed lymphocytes and showed almost no fatty replacement. The spleen contained numerous, unusually large malpighian bodies with well developed germinal centers, while the liver showed large numbers of lymphocytes in the portal areas. The enlargement of the lymph nodes was seen to be due to marked hyperplasia of the reticulum cells, which occupied mainly the sinuses of the medulla.

*Adrenal Glands.*—These glands were not identified in situ, but a prolonged search of the regional tissues, after a period of fixation, was rewarded by the finding of a small roughly triangular body on each side. These bodies were of almost identical appearance, each measuring 11 by 5 by 2 mm. Together they weighed 1.0 Gm. Each was composed of what appeared to be a fibrous capsule

enclosing a thin layer of softer grayish tissue. No accessory adrenal tissue was found.

Microscopically, the two adrenal glands resembled each other so closely that the following description is applicable to both. Each was composed entirely of medullary tissue surrounded by a thickened capsule; no vestige of cortex was present in the many sections examined. The striking abnormality of the medulla was the extreme degree of lymphocytic infiltration. For the most part, the infiltrating cells were so closely packed that at first glance the gland resembled a lymph node, and it was only after closer scrutiny that parenchymal cells were seen. In a few areas the adrenal tissue was entirely free of cellular infiltration and relatively normal in appearance, and here, as in other areas where the lymphocytic collections were less dense, numerous chromaffin cells were visible (fig. 3 A). The latter were of the usual polygonal shape and showed no degenerative change. An occasional nonmyelinated nerve and a few large ganglion cells were present, while the normal-appearing central vein with its characteristic thick muscle bundles was readily identified. The fibrous tissue comprising the capsule was uniformly increased in amount, but it was not possible to be sure whether this represented a proliferation of capsular connective tissue or merely collapse of the cortical stroma. Apart from slight thickening, the capsular and pericapsular blood vessels showed no abnormality.

#### CASE 7

*History.*—A 33 year old white woman, unmarried, was admitted to the Women's College Hospital, Toronto, May 15, 1939. She complained of weakness, fainting attacks and vomiting of two months' duration. There was brown pigmentation of exposed areas and of the buccal mucosa. The blood pressure was 84 systolic and 70 diastolic. A diagnosis of Addison's disease was made, and three months later the patient was discharged improved, to continue with the parenteral administration of extract of adrenal cortex and oral salt therapy. The extract was later replaced by desoxycorticosterone acetate. On this regimen she remained fairly well but never became strong enough to work again as a stenographer. She was readmitted to the hospital Feb. 2, 1940 with pneumonia, from which she made a slow recovery. In May one 150 mg. pellet of desoxycorticosterone acetate was implanted in the subcutaneous tissues of the left infrascapular region. At this time the blood pressure was in the neighborhood of 92 systolic and 68 diastolic. The final admission was on Feb. 26, 1941. The patient complained of severe weakness for three weeks and of vomiting and fainting during the morning of the day of admission. Nasopharyngitis was present, and later hyperpyrexia developed. Despite intravenous therapy and large doses of aqueous extract of adrenal cortex, death occurred five days later.

*Autopsy* (two hours after death at the Women's College Hospital by Dr. V. Laughlen).—The body was normally developed and fairly well nourished and showed diffuse brownish pigmentation of the skin. The thymus gland was not identified, and the lymph nodes were not enlarged. The ovaries were normal in size and appearance, as was the heart, which weighed 225 Gm. The spleen weighed 170 Gm. and was likewise normal. A small accessory spleen was present. The thyroid gland and the cranial contents were not examined. The other organs apart from the adrenal glands showed no lesions.

Microscopically, there were no additional observations. With the exception of the right adrenal gland, none of the organs showed lymphocytic infiltration.

*Adrenal Glands.*—These glands were identified with difficulty because of their small size. The left was slightly larger than the right, but the two together made

up not more than a small fraction of a normal gland. No golden brown cortical tissue was visible in either, the cut surfaces of each being of a homogeneous dull grayish color.

Microscopically, no cortical tissue remained on the left side, the gland being made up of medullary tissue enclosed by a normally thin capsule. The medulla, which did not seem to be reduced in amount, contained a normal central vein, a

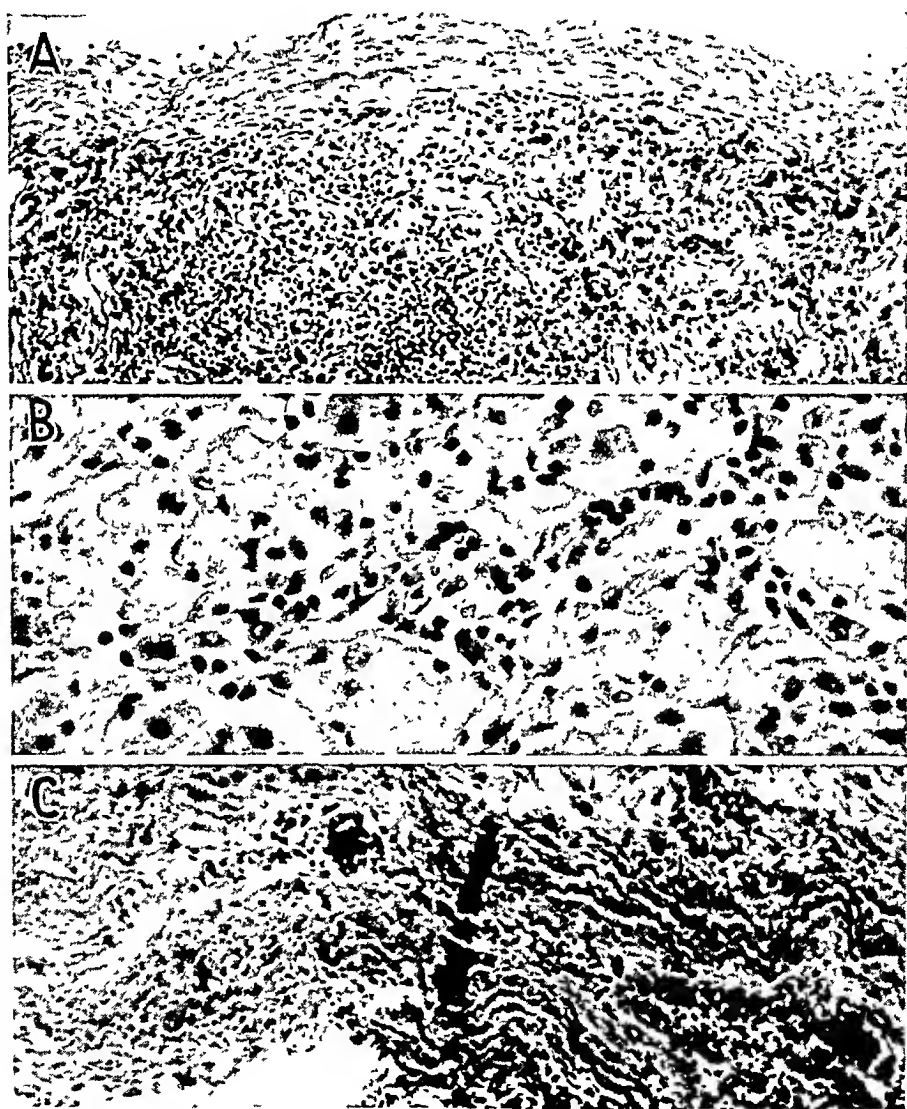


Fig. 3.—*A*, absence of cortical tissue and extreme degree of lymphocytic infiltration of the medulla in case 6. Hematoxylin and eosin;  $\times 110$ . *B*, degeneration and necrosis of cortical cells in case 7. Hematoxylin and eosin;  $\times 250$ . *C*, capsule of the left adrenal gland (case 8)—all that remains of this gland. Hematoxylin and eosin;  $\times 200$ .

few nonmedullated nerves and ganglion cells. The individual cells of the medulla, although not altered in appearance, seemed to be somewhat reduced in number. Thin-walled vascular channels were more prominent than in a normal gland. There was neither fibrosis nor lymphocytic infiltration. The right gland showed small

numbers of cortical cells at one end but no other cortical tissue. No vestige of normal cortical structure remained. The few cortical cells were separated by collapsed stroma and without exception showed severe degenerative changes or actual necrosis. The nuclei, for the most part, were pyknotic and misshapen, and the cell boundaries were indistinct. There was diffuse lymphocytic infiltration of this residual cortical tissue (fig. 3 B). In the medulla scarcity of parenchymal cells, increase in fibrous tissue and rather dense lymphocytic infiltration were apparent. The capsule was moderately thickened, and in some situations only a few strands of loose connective tissue and focal collections of lymphocytes intervened between its two layers.

#### CASE 8

*History.*—A 16 year old white youth was first admitted to the Toronto General Hospital in December 1937. His complaints were weakness, loss of appetite and intermittent attacks of nausea and vomiting since starting to work some five months previously. Examination revealed a slightly built, emaciated and mentally dull adolescent male. The other significant findings included persistent hypotension (the blood pressure varying from 52 systolic and 25 diastolic to 100 systolic and 70 diastolic), a slight degree of diffuse brownish pigmentation of the skin of the forehead and the elbows, and a blood sodium content of 270 mg. per hundred cubic centimeters. The tuberculin test was negative. Roentgen plates of the adrenal regions showed no abnormality. A diagnosis of Addison's disease due to simple atrophy of the adrenals was made. With large doses of sodium chloride by mouth and aqueous extract of adrenal cortex intramuscularly, the patient showed marked improvement and gain in weight. He was discharged in April 1938 and continued to take the salt and the extract. In September he was readmitted in a severe addisonian crisis precipitated by a cold in the head and sore throat. The condition was controlled by intravenous injections of hypertonic saline solution and with extract of adrenal cortex, which was later replaced by desoxycorticosterone acetate. The patient carried on, feeling moderately well but not strong enough to work, until January 1940, when he was admitted to the hospital in a semi-comatose state with nasopharyngitis. The blood sugar amounted to 27 mg. per hundred cubic centimeters, and a diagnosis of hypoglycemic reaction was made. This was controlled satisfactorily by intravenous administration of dextrose. A similar attack occurred in July 1941, when, following the development of a sore throat, the patient missed two or three meals. Shortly thereafter he was admitted to the hospital unconscious, with a blood sugar determination of 33 mg. per hundred cubic centimeters. Consciousness was promptly regained following intravenous administration of dextrose, and the patient was discharged a few days later. Three days before the final admission he experienced general malaise and loss of appetite, and early in the morning of the day of admission (November 18) he was found in an unconscious state. On admission he was moribund, and signs of bronchopneumonia were present. Death occurred a few hours later.

*Autopsy* (three hours after death).—The cadaver was that of a well developed but emaciated youth. The pigmentation, which was not marked, corresponded to that which had been observed clinically. The axillary and the pubic hair were sparse, but the genitalia showed normal development. The lungs were heavier than normal, and clear fluid could readily be expressed from their cut surfaces. The lower lobe of the right lung contained scattered small areas of pneumonic consolidation. The thyroid gland, which weighed 25 Gm., was somewhat tougher and more fibrous than normally. The thymus gland was a bilobed mass of firm pinkish white tissue, weighing 15 Gm. The abdominal lymph nodes were definitely

enlarged, pale, soft and succulent. They measured up to 2 cm. in greatest diameter. The pituitary gland was normal on gross examination and weighed 0.5 Gm. The brain was edematous and increased in weight. Apart from the adrenal glands, the other organs, including the heart and the spleen, which weighed 260 and 225 Gm., respectively, were normal.

Microscopically, the lungs showed marked confluent bronchopneumonia involving both lower lobes and edema and patchy small areas of pneumonic change in the upper lobes. The thyroid gland exhibited marked fibrosis; broad bands of scar tissue separated individual lobules and parts of lobules, the component acini of which were formed by flattened cells and contained much colloid. Extremely large and numerous areas of lymphoid tissue replacement of thyroid tissue proper were seen. In these situations there were closely packed lymphocytic collections, measuring up to 1 mm. in diameter. Some of these showed "germinal centers." The thymus gland showed only slight fatty replacement and, the patient's age being taken into consideration, was definitely hyperplastic. The only notable finding in the liver was moderate infiltration of the portal areas with lymphocytes. The abdominal and the thoracic lymph nodes presented a picture of "sinus catarrh," the cortical and the medullary sinuses being filled with proliferating reticulum cells. In the perineural sheaths of some of the larger retroperitoneal nerves there were closely packed lymphocytes, although there was no suggestion of any inflammatory or degenerative lesion involving these structures. The testes showed normal spermatogenesis and some degree of hyperplasia of the interstitial cells, moderately large islets of these cells being scattered at intervals between the tubules. The interstitial connective tissue was somewhat increased in amount. The pituitary gland presented diffuse fibrosis of the anterior lobe, with considerable diminution in the number of parenchymal cells, so that the volume of secretory tissue was significantly reduced. The normal ratio between chromophobe and acidophil cells was not altered, but basophil cells were infrequently encountered and were practically confined to the extreme posterior part of this lobe. The posterior lobe was normal. Sections of the brain confirmed the presence of edema. A section of abdominal skin showed an increased number of melanophores in the cutis vera and a slight increase of the melanin content of the basal layer of the epidermis. The other organs, including the heart, the kidneys, the spleen and the pancreas, were histologically normal.

*Adrenal Glands.*—Despite an intensive search with the retroperitoneal organs in situ, no trace of adrenal tissue was found. The aorta was laid open, and the adrenal arteries on the two sides were identified with difficulty because of their diminutive size. It was possible to trace them for only a short distance into the periadventitial areolar tissue of the aorta, where they disappeared and could be followed no farther. No adrenal branch could be demonstrated arising from either the inferior phrenic or the renal artery on either side. A block of tissue extending from the lower part of the thoracic aorta to the origin of the inferior mesenteric artery and including the diaphragm, the kidneys, the adrenal fatty tissues, the inferior vena cava and all the fatty areolar tissue of the fossae renales and upper lateral abdominal gutters was then taken. This was painstakingly dissected on repeated occasions for a period totaling six hours without any grossly recognizable adrenal tissue being encountered. A small flattened scarred area, the component tissues of which had a volume of approximately 0.5 cc., was encountered in the adrenal region on each side, and both of these were preserved for serial microscopic sectioning in the belief that they probably represented remnants of the adrenal glands.



Microscopically, the thickened area from the right side was found to be only patternless scar tissue. No trace of adrenal tissue was identified in it or in any of the blocks taken from that side. The area of thickening from the left side proved to be all that remained of the left adrenal. Only the wavy, somewhat thickened capsule persisted, but it outlined unmistakably the characteristic contour of an adrenal gland (fig. 3 C). Serial microscopic sections showed no parenchymal tissue of either cortical or medullary type; only fatty areolar tissue containing small blood vessels and occasional scattered lymphocytes was present within the fibrous envelope of the gland.

#### COMMENT

Clinically, these cases are noteworthy for their remarkable similarity in regard to symptoms and disease progress. Six of the 8 patients were women; the youngest was 20 and the oldest 44 at the time of death. All the patients suffered from gradually developing and slowly progressive weakness and asthenia interspersed with attacks of prostration and vomiting. All had some degree of hypotension, and pigmentation developed in all. All died in an Addisonian crisis between two and four years after the onset of symptoms.

*Adrenal Lesions.*—The lesions encountered in the adrenal glands presented the same striking similarity from case to case as did the clinical course of the patients. No parallelism could be found, however, between the duration of symptoms and the severity of the lesions.

In cases 1 and 8 the process had reached an end stage; no glandular tissue was found. It is possible that in case 1 a more painstaking search would have revealed remnants of the adrenal gland, especially so in view of the difficulty with which adrenal tissue was found in several of the other cases. The experience in case 8, however, in which a most exhaustive search demonstrated that all glandular tissue had disappeared, as well as the failure of several other observers<sup>3</sup> to find persisting glandular tissue, inclines me to the belief that in case 1, as in case 8, no glandular tissue existed at the time of death.

In the other 6 cases the significant abnormalities involved the cortex. In cases 4 and 6 there was complete absence of cortical tissue. In case 7 the amount of residual cortex was limited to a few isolated cells, while in cases 2, 3 and 5, although cortical tissue was readily identified, the destructive process was extremely far advanced, the viable tissue having been reduced to a small fraction of the normal. In these 4 cases in which cortical tissue remained, the picture was one of degeneration and necrosis with resultant reduction in cortical volume. In the other 2 cases the process of cortical destruction had reached an end stage; only collapsed cortical stroma intervened between the wrinkled capsule and the medullary tissue. Nothing was found to suggest that the lesions

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3. Brenner, O.: *Quart. J. Med.* **22**:121, 1928 (table summarizing cases at conclusion of article).

might have been of ischemic or tuberculous origin. A consistent feature of all cases except 1 and 8, which have been considered separately, was the relatively close resemblance of the one adrenal to its fellow of the opposite side, each containing approximately the same amount of residual cortex. An additional constant finding, which has been frequently noted by previous writers,<sup>4</sup> was the marked degree of lymphocytic infiltration of the medulla and of the degenerate cortical tissue. In contrast to the cortex, and despite the lymphocytic infiltration, the medulla was relatively well preserved and contained probably not far from its normal complement of chromaffin cells. This marked loss of cortical tissue with preservation of the medulla in 6 of the 8 cases is in agreement with the accumulating clinical and experimental evidence which indicates that Addison's disease from any cause is the result of cortical damage and that damage to the medulla plays at most a minor role in its production.

A study of the process which results in the great reduction in volume of cortical tissue indicates that it is clearly not one of simple atrophy in the restricted sense of the term, for although the gland is unquestionably smaller than normal, it does not shrink because of atrophy of its individual cells but because of destruction and disappearance of these cells. Neither is the process hypoplasia as has been suggested by some writers; the degenerative changes in the cortical cells, together with the wrinkling of the capsule and the disruption of the cortical structure, point to an acquired lesion. Further, as Guttman<sup>4a</sup> has indicated, it is extremely difficult to conceive of a congenital underdevelopment of which no symptoms are present until as late as the fourth or the fifth decade. Although the almost invariable finding of lymphocytic infiltration of the adrenal glands in the present and other series of cases might suggest that the process is inflammatory, no other feature suggestive of inflammation is found. Areas of lymphocytic infiltration in otherwise normal adrenal glands are encountered by no means infrequently in routine autopsies. Moreover, collections of lymphocytes such as those present in the adrenal glands in the cases under consideration, when seen, as they so commonly are, in such conditions as nephrosclerosis and hepatic cirrhosis, are not interpreted as being inflammatory in origin but rather as accompanying the process of degeneration. Although this progressive and bilateral destructive process involving especially the adrenal cortex suggests the action of some damaging substance or substances transported by the blood stream, the nature of the hypothetic agents is completely unknown. An analogy has been drawn by Wells<sup>4b</sup> between the destructive process involving the adrenal cortex in cases of "adrenal atrophy" and diffuse toxic necrosis of the liver. Indeed, apart from an

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4. (a) Guttman, P. H.: *Arch. Path.* **10**:742 and 895, 1930. (b) Wells, H. G.: *ibid.* **10**:499, 1930. Susman.<sup>2b</sup>

apparent dissimilarity in tempo, the two processes seem to differ in no essential respect. In the same sense as the term "subacute necrosis of the liver" is preferable to "subacute yellow atrophy," so is the term "necrosis of the adrenal cortex" preferable to the term "adrenal atrophy."

*Associated Findings.*—Several reports<sup>5</sup> have indicated that a small heart is to be expected in cases of Addison's disease, especially in those in which the disease is due to "atrophy." In the 7 cases in which the heart was examined, the average weight was 220 Gm., a weight not less than that to be expected in any case of prolonged wasting disease. The other organs, generally speaking, were smaller than usual, in keeping with the patients' state of poor nutrition.

The majority of articles dealing with Addison's disease from the standpoint of pathology have recorded varying degrees of hyperplasia of lymphoid tissue throughout the body. In all cases in the present series with the exception of case 7 there was some degree either of lymphoid hyperplasia or of lymphoid infiltration of organs. There was generalized hyperplasia of lymph nodes in case 6, together with non-involution of the thymus gland and enlargement of Peyer's patches. In cases 3 and 8 also the thymus gland was large and hyperplastic, while in cases 4 and 8 many of the lymph nodes were considerably increased in size. Enlargement of the malpighian bodies of the spleen was seen in 3 cases. Lymphocytic infiltration of organs, described by Duff and Bernstein,<sup>2a</sup> was observed in the present series. In 2 cases the organs involved were the kidneys, and 1 of these cases showed in addition round cell infiltration of the meninges. In another case there was lymphocytic infiltration of the portal areas of the liver and of the sheaths of several of the retroperitoneal nerves. In all 5 cases in which the thyroid gland was examined, it exhibited large, well developed lymph nodules with prominent, active-appearing germinal centers. The significance of these findings is difficult to evaluate, the more so because of the relatively frequent occurrence of one or more of them in the same case in routine autopsy material.

Of the 5 thyroid glands examined, only 1 was of average size. The other 4 were small, 2 weighing 20 Gm. each and the 2 others 15 and 10 Gm., respectively. Microscopically, the striking lesion in all 5 was the remarkable degree of lymphoid infiltration and lymphoid hyperplasia. In 1 case these features were so marked as to suggest at first sight a lymphadenoid goiter. An additional constant feature was fibrosis and fibrous tissue replacement of parenchymal elements. In no instance was there any sign of epithelial hyperplasia, the acini being for the most part small, lined by low cuboidal or flattened epithelium and filled with

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5. Susman,<sup>2b</sup> Brenner.<sup>3</sup>

homogeneous deep-staining colloid. These characteristics of the thyroid gland in Addison's disease—atrophy, lymphoid infiltration and fibrosis—have been previously remarked on by Brenner,<sup>3</sup> Susman<sup>2b</sup> and others.

The pituitary gland was grossly normal in all 4 cases in which it was examined, and in 1 of these cases no microscopic abnormality was found. In the other 3 cases (3, 4 and 8), however, similar lesions were presented: a diffuse increase of fine connective tissue in the anterior lobe and a great scarcity of basophil cells, the latter being represented by an occasional cell at the extreme posterior end of the pars glandularis. In cases 3 and 4 these few remaining basophil cells showed nuclear pyknosis and vacuolation of the cytoplasm, indicating a degenerative process. The chromophobe and eosinophilic cells appeared to be reduced in numbers in these cases as well as in case 8. In none of the 4 cases was a differential cell count made, although in each case granule stains were employed. The increased connective tissue content and the diminution in the number of basophil cells are in agreement with the findings of Crooke and Russell<sup>6</sup> in their study of the pituitary glands in a series of cases of Addison's disease. The abnormal transitional forms described by these workers, however, were not recognized in the present material.

In 1 case the fat tissue of the body was described as being of a peculiar dark yellowish brown color, and this same unusual pigmentation of fat, a finding which to my knowledge has not been previously recorded in this condition, was seen in 3 of the last 4 cases of Addison's disease due to adrenal tuberculosis coming to autopsy in this department. At the time of autopsy the possibility that the coloration was due to carotene or an allied fat-soluble pigment was considered. Despite the fact that several cases of carotenemia must have come to autopsy in this department not one member of the department recalled having previously seen fat tissue comparable in color with that observed in these cases as would be expected if the pigment in this instance had been carotene. Analysis of portions of subcutaneous and abdominal fat tissue in the 3 cases of Addison's disease due to tuberculosis mentioned revealed only a slightly higher than average carotene and carotenoid content. In view of the evidence that the pigmentation was probably due neither to carotene nor to a fat-soluble substance of similar nature, the possibility that it may have resulted from the deposition of melanin in low concentration must be kept in mind. No investigation of the tissue was carried out with a view to determining its possible content of melanin.

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6. Crooke, A. C., and Russell, D. S.: *J. Path. & Bact.* **40**:255, 1935.

## SUMMARY

Eight cases of Addison's disease due to a nontuberculous destructive process of the adrenal cortex are reported. In 6 cases the lesion was one of cortical destruction, with severe and widespread necrosis of the cortex in 4 cases and complete disappearance of cortical tissue in the other 2. In each of these 6 cases the adrenal medulla was comparatively well preserved but constantly showed lymphocytic infiltration. In 2 additional cases no adrenal parenchymal tissue remained, although the process in these cases was presumed to be of the same nature as that in the other 6.

The thyroid gland was atrophic, fibrosed and infiltrated with lymphocytes. The pituitary gland in 3 of 4 cases in which it was examined showed a diffuse increase in the connective tissue of the anterior lobe and a great scarcity of basophil cells. The eosinophilic and chromophobe cells were also reduced in number.

A hitherto unrecorded pigmentation of fat tissue is reported.

The findings indicate that the process in the adrenal glands is not one of true atrophy, a term under which cases of this nature have popularly been described, but rather one of disappearance of tissue consequent on cortical necrosis. No clue as to the cause of the cortical necrosis was obtained.

The incidence of this pathologic process in the present series of cases of Addison's disease was 41 per cent.

# STUDIES OF NORMAL AND OF ABNORMAL MITOTIC ACTIVITY

## II. THE RATE AND THE PERIODICITY OF THE MITOTIC ACTIVITY OF EXPERIMENTAL EPIDERMOID CARCINOMA IN MICE

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Autonomy of growth is one of the principal characters of cancer. Growth in both neoplasms and normal tissues is due principally to mitotic cellular division. The rate and the periodicity of the mitotic activity of normal tissue are ultimate expressions of the factors which regulate that activity. A comparison of cancer and the normal tissue from which it is derived with respect to rate and periodicity of mitotic activity may shed light on certain fundamental characters of the cancer cell.

The literature reveals a single study along this line. Dublin, Gregg and Broders<sup>1</sup> removed two specimens of cancerous growth in each of 5 cases of carcinoma of the large intestine of man, one between 10 a. m. and 12 m., and one between 10 and 12 p. m. In each specimen they counted 3,000 to 5,000 tumor cells and established as a mitosis coefficient the number of mitoses per thousand of tumor cells. The mitosis coefficients with the exception of that for one specimen were essentially the same morning and night. They deduced that mitotic rhythm was absent. No control specimens were examined. This deduction is not warranted, for it is based on the assumption that the mitotic activity of colonic epithelium duplicates that of human epidermis, which has been shown to be minimum in the morning and maximum at night.<sup>2</sup> The curve of mitotic activity for one tissue of an organism is not necessarily similar to that for another; in fact, the curves for rat renal cortex, sub-maxillary salivary gland and epidermis are quite different.<sup>3</sup> In human colonic epithelium maximum and minimum mitotic activity may well occur at times other than 10 a. m. to 12 m. and 10 to 12 p. m.

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From the Department of Pathology of the Cleveland City Hospital and the Institute of Pathology of Western Reserve University.

1. Dublin, W. B.; Gregg, R. O., and Broders, A. C.: Proc. Staff Meet., Mayo Clin. **15**:623, 1940; Arch. Path. **30**:893, 1940.

2. Cooper, Z. K., and Schiff, A.: Proc. Soc. Exper. Biol. & Med. **39**:323, 1938. Cooper, Z. K.: J. Invest. Dermat. **2**:289, 1939. Broders, A. C., and Dublin, W. B.: Proc. Staff Meet., Mayo Clin. **14**:423, 1939.

3. Blumenfeld, C. M.: Arch. Path. **33**:770, 1942.

If deductions concerning the rate and the rhythm of mitotic activity in tumors are to be valid, certain variables must be controlled as rigidly as possible. They include the type, differentiation, location, size and age of the tumor and the species, strain, sex and age of the host. The use of tumors experimentally produced in animals permits a degree of control of these variables practically impossible of attainment in man.

#### MATERIALS AND METHODS

Mice of the CBA strain, bred from animals provided by Dr. L. C. Strong, of the department of anatomy of Yale University, were used because the incidence of spontaneous tumors in them is negligible. The carcinogen was methylcholanthrene, applied as a 0.3 per cent solution in benzene. The experiment was begun with 65 male mice 2 months old. The solution was applied with a no. 6 camel's hair brush, two strokes being made twice weekly in the interscapular region. In fourteen to fifteen weeks, with remarkable regularity, grossly discernible tumors, which proved to be cancerous, appeared in most of the mice. Usually there were several cancers as well as benign papillomas in the painted area. As a check on the nature of the apparent cancers, 1 mouse from each of 6 litters was set aside at the end of fourteen or fifteen weeks. The lesions in each of these mice without further application of carcinogen progressed, coalescing or remaining separate, to form single or multiple large ulcerated masses, the seat of extensive necrosis and suppuration. The remaining animals were observed daily. When a tumor judged grossly to be a cancer reached a maximum diameter of 1 cm., the animal bearing it was killed. The average interval from the first application of the carcinogen to autopsy was one hundred twenty-three and seven-tenths days. The entire painted area and a piece of normal skin from the ventral central abdominal region were removed, fixed at once in Bouin's fluid, embedded in paraffin, sectioned at 8 microns and stained with hematoxylin and eosin. Animals were killed at 8 a. m., 12 m., 4 p. m., 8 p. m., 12 p. m. and 4 a. m. Members of a litter were distributed as evenly as possible through the twenty-four hour period to minimize the effect of inherent differences in mitotic activity. In this manner test and control specimens were obtained from 60 mice, ten for each period of time. The tumors used were all diagnosed as well or moderately well differentiated squamous cell carcinoma. They showed partial or complete lack of cell stratification and irregular keratinization, and some of them, loss of intercellular bridges. Pleomorphism of cells was moderate to marked, with occasional giant multinucleated forms. There were numerous abnormal mitoses, including multipolar, unequal and widely dispersed forms. All tumors showed invasion of the dermis and direct extension into the subcutaneous layer; many, direct extension into or through the panniculus carnosus. Neither regional nor remote metastasis was observed, which may have been due to the nature or to the short period of existence of the tumor or to both. Degeneration, necrosis, ulceration and suppuration of the tumors were common, and such areas were avoided in counting mitoses. Occasionally, fibrosarcoma developed in juxtaposition to the carcinoma, presumably because ulceration afforded the carcinogen access to the dermis.

Mitotic activity was determined by counting the mitoses in 500 fields, as explained in a previous article.<sup>3</sup> Each field measured 60 by 70 microns. The sections were cut perpendicular to the cutaneous surface, at a thickness of 8 microns. Sections of tumor readily afforded 500 fields, each 60 by 70 microns. Sections of

normal epidermis did not fill the field. Instead, 500 segments of epidermis, each 60 microns long, 8 microns thick and of varied width, were studied. Therefore, the volume of tumor studied was greater than that of epidermis. Provided the sample of each specimen studied was representative of the whole, this difference in volumes will not affect conclusions concerning the presence or the absence or the character of the diurnal variation or of the periodicity of the mitotic activity of the normal and of the cancerous epidermis; it will, however, make impossible a comparison of the rates of the mitotic activity of normal epidermis and the tumor. For such a comparison a determination of the rate of mitosis per cell would be best, but there are certain difficulties of technic and interpretation which deserve mention. Indistinct cell limits would necessitate counting nuclei rather than cells in normal epidermis and in carcinoma, which would result in a determination of the rate of mitosis per nucleus. Enumeration of each nucleus of multinucleated tumor cells would introduce an error. Keratinized cells of normal epidermis do not exhibit mitosis; yet it is not possible to distinguish clearly epidermal cells capable of undergoing mitotic division, which should, therefore, be counted, from cells in which mitosis cannot occur. Factors such as the production of less or more than two daughter cells by abnormal mitosis, the duration of mitosis, the length of the intermitotic interval and the life period of a cell, all of which should be considered, cannot be determined from the material used in this study. Because of these difficulties it was decided to express rate of mitosis as the number counted in 500 fields. For the tumor, this amounted to an expression of the number of mitoses in 500 portions, each 60 by 70 by 8 microns, or 0.0168 cu. mm. of tumor. For normal epidermis, the volume varied with the width of epidermis in each specimen. The average width was determined for each specimen by measuring every fiftieth field counted, with an ocular micrometer. The volume of the observed portion of each specimen of normal epidermis was calculated, and from this and the number of mitoses counted, an estimate was made of the number of mitoses in a volume equal to the volume of tumor studied. For example, in the specimen of normal epidermis of mouse F2 the average width of the epidermis was 7 microns. The volume of epidermis studied was 500 (60 by 8 by 7 microns) cubic microns or 0.00168 cu. mm. The number of mitoses observed was 10. The volume of the epidermis being one tenth of the volume of the tumor studied, multiplication of the observed number of mitoses by 10 permits a comparison of the numbers of mitoses in equal volumes of epidermis and tumor. The values thus calculated, i. e., the numbers of mitoses in volumes of epidermis equal to the volumes of tumor, are referred to as corrected values. Fisher's<sup>4</sup> small sample methods have been employed in the statistical analysis of the data obtained. In tables 2 and 3, containing statistical data, the symbol *t* is a significance ratio. With it, *P* is determined according to degrees of freedom. *P* is an expression of the likelihood of occurrence of a difference the same as that observed by chance. A *P* of 0.01 means this chance is 1 of 100; a *P* of 0.6, 60 of 100. Conventionally, a *P* of 0.05 or less is interpreted as indicating statistical significance.

## RESULTS

*Normal Epidermis.*—The averaged values listed in table 1 and represented as a curve in the accompanying chart show a diurnal rhythm of mitotic activity. Maximum mitotic activity occurred at 8 a. m. to

4. Fisher, R. A.: Statistical Methods for Research Workers, ed. 7, London, Oliver & Boyd, 1938.



TABLE 1.—*Individual and Average Numbers of Mitoses per Five Hundred Fields*

8 a. m.	12 m.	4 p. m.	8 p. m.	12 p. m.	4 a. m.
For Epidermis					
22	75	30	18	8	15
7	64	45	22	11	20
32	6	13	1	8	23
13	37	20	18	8	6
17	57	6	1	6	11
14	10	2	8	2	12
10	28	6	9	2	6
10	11	1	3	10	6
28	27	13	1	2	19
24	59	9	10	2	6
17.7	37.4	16.5	9.1	5.9	12.4
Corrected Values for Epidermis					
97	338	156	67	62	77
59	384	167	121	63	112
365	67	86	7	87	129
142	278	136	131	69	51
156	342	46	13	55	90
129	74	152	55	21	82
100	314	61	76	25	55
109	99	15	32	109	55
274	232	122	8	18	158
266	325	82	89	16	59
169.7	245.3	102.3	59.9	53.0	86.8
For Carcinoma					
93	260	127	96	123	155
214	136	201	335	341	159
109	115	212	176	149	222
169	222	192	177	164	233
131	371	226	168	263	255
526	262	257	144	190	223
114	294	205	272	221	170
265	204	258	304	177	188
228	193	260	184	220	211
242	352	138	201	225	339
200.1	240.9	207.6	214.7	207.8	215.5

TABLE 2.—*Statistical Data on the Significance of the Difference Between the Average Values of Mitotic Activity at Various Periods of Time*

Times Compared	Corrected Values for Epidermis			Carcinoma		
	Diff. $\pm$ S. E.	t	P	Diff. $\pm$ S. E.	t	P
8 a.m. vs. 12 m.	75.6 $\pm$ 49.3	1.532	0.2-0.1	31.8 $\pm$ 48.2	0.660	0.6-0.5
8 a.m. vs. 4 p.m.	67.4 $\pm$ 35.2	1.917	0.1-0.05	1.5 $\pm$ 42.8	0.035	0.9
8 a.m. vs. 8 p.m.	109.8 $\pm$ 34.2	3.269	0.01	5.6 $\pm$ 47.4	0.118	0.9
8 a.m. vs. 12 p.m.	116.7 $\pm$ 32.7	3.569	0.01	1.3 $\pm$ 44.9	0.028	0.9
8 a.m. vs. 4 a.m.	82.9 $\pm$ 33.1	2.505	0.05-0.02	6.4 $\pm$ 43.7	0.146	0.9-0.8
12 m. vs. 4 p.m.	143.0 $\pm$ 41.1	3.475	0.01	33.3 $\pm$ 30.5	1.093	0.3-0.2
12 m. vs. 8 p.m.	185.4 $\pm$ 40.9	4.535	0.01	26.2 $\pm$ 36.6	0.716	0.5-0.4
12 m. vs. 12 p.m.	192.3 $\pm$ 39.6	4.858	0.01	33.1 $\pm$ 33.2	0.996	0.4-0.3
12 m. vs. 4 a.m.	158.5 $\pm$ 39.9	3.963	0.01	25.4 $\pm$ 31.4	0.809	0.5-0.4
4 p.m. vs. 8 p.m.	42.4 $\pm$ 21.7	1.950	0.1-0.05	7.1 $\pm$ 29.1	0.243	0.9-0.8
4 p.m. vs. 12 p.m.	49.3 $\pm$ 19.3	2.557	0.02-0.01	0.2 $\pm$ 24.8	0.008	0.9
4 p.m. vs. 4 a.m.	15.5 $\pm$ 19.5	0.796	0.5-0.4	7.9 $\pm$ 22.8	0.347	0.8-0.7
8 p.m. vs. 12 p.m.	6.9 $\pm$ 17.5	0.393	0.7-0.6	6.9 $\pm$ 32.0	0.215	0.9-0.8
8 p.m. vs. 4 a.m.	26.9 $\pm$ 18.3	1.470	0.2-0.1	0.8 $\pm$ 30.5	0.026	0.9
12 p.m. vs. 4 a.m.	33.8 $\pm$ 15.2	2.216	0.05-0.02	7.7 $\pm$ 26.0	0.296	0.8-0.7

Diff. = Difference between average values.

S. E. = Standard error of the difference between average values.

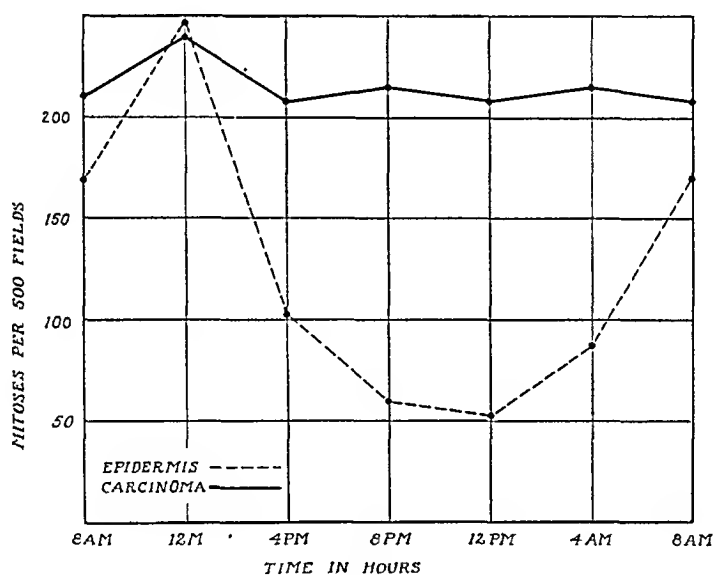
t = Significance ratio, obtained by dividing the difference between average values by the standard error of the difference between average values.

P = An expression of the likelihood of occurrence by chance of a difference the same as that observed. It is obtained from t according to the number of cases in the series compared. P of 0.01 means this chance is 1 of 100; P of 0.6, 60 of 100. Conventionally, P of 0.05 or less is interpreted as indicating statistical significance.

12 m.; minimum mitotic activity, at 8 p. m. to 12 p. m. The corrected values for epidermis show more than four times as many mitoses at noon as at midnight. The statistical studies recorded in table 2 show that the difference is significant. The character of the curve is similar to

TABLE 3.—*Comparison of Mitotic Activity in Epidermis (Corrected Values) with Mitotic Activity in Carcinoma*

Time	Epidermis vs. Carcinoma		Diff. $\pm$ S. E.	t	P
8 a.m. ....	169.7	209.1	$39.4 \pm 50.8$	0.775	0.9
12 m. ....	245.3	240.9	$4.4 \pm 46.6$	0.094	0.9
4 p.m. ....	102.3	207.6	$105.3 \pm 22.1$	4.764	0.01
8 p.m. ....	59.9	214.7	$154.8 \pm 28.9$	5.358	0.01
12 p.m. ....	53.0	207.8	$154.8 \pm 22.4$	6.917	0.01
4 a.m. ....	86.8	215.5	$128.7 \pm 20.7$	6.215	0.01



Mitotic activity at four hour intervals for a period of twenty-four hours in experimental epidermoid carcinoma and in an equal volume of normal epidermis. Average numbers of mitoses were plotted.

that obtained for rat epidermis,<sup>3</sup> and the demonstration of periodicity is in agreement with the findings of Ortiz Picón<sup>5</sup> and Cooper and Franklin.<sup>6</sup> Carleton<sup>7</sup> also demonstrated periodic mitotic activity in mouse epidermis, but the maximum and minimum periods were at opposite times. No explanation is available for this difference.

*Carcinoma.*—Individual values and the averages are listed in table 1, statistical data in tables 2 and 3 and the curve of the averages in the chart. First, it should be noted, *there was no diurnal rhythm.* The

5. Ortiz Picón, J. M.: Ztschr. f. Zellforsch. u. mikr. Anat. **23**:779, 1933.

6. Cooper, Z. K., and Franklin, H. C.: Anat. Rec. **78**:1, 1940.

7. Carleton, A.: J. Anat. **68**:251, 1934.

minor variations between averages when these are compared in every possible combination are without any statistical significance. The average mitotic activity at 8 a. m., 4 p. m., 12 p. m. and 4 a. m. is almost identical. Individual values of mitotic activity for carcinoma show much less variation than those for epidermis. Second, *the mitotic activity in the carcinoma was no greater than the maximum mitotic activity in an equal volume of normal epidermis.* At 8 a. m. and 12 m. there was no significant difference in average number of mitoses between carcinoma and normal epidermis. During the remainder of the twenty-four hour period, as mitotic activity remained constant in the tumors and waned and waxed in the normal epidermis, the differences were statistically significant.

#### COMMENT

The application of these results to neoplasms in general is contingent on the demonstration of similar results in other cancers, at different ages and in various species. The observations of Dublin, Gregg and Broders<sup>1</sup> are inadequate to furnish support. Therefore, this discussion must be predicated on the assumption that the results are generally true.

A neoplasm is an autonomous or independent growth. This implies that normal tissue growth is dependent. The demonstration of periodicity of mitotic activity is evidence of control of growth. Periodicity being different for each of three organs studied in the rat,<sup>3</sup> it is probable that the factors directly concerned in the daily regulation of growth are not central, not within the cell, but in the organ. It must be stressed that attention is here directed toward the factors which daily regulate growth. Heredity, hormones, nutrition, the nervous system, all play their part. However their manifold influence may be integrated, whatever their role may be in affecting mitotic activity, the organ is apparently the unit through which these factors may act on the cell.

A normal cell as the constituent of an organ has two types of activities: vegetative and functional. Among the vegetative activities is cell division. The functional activities are the result of differentiation and organization of cells. It has long been supposed that there is a partition of labor in the cell. It may be hypothesized that as a cell differentiates to become part of an organ it develops a component specifically affected by the functional stimuli peculiar to the organ of which it is a part—a component responsible for the shift between vegetative and functional activity in the cell. Thus, the cells of each organ may be supposed to have a characteristic component. In the submaxillary salivary gland it would be affected by the influences stimulating the secretion of saliva; in the kidney, by influences stimulating the secretion of urine. When affected, it would shift cell activity from vegetative to functional.

On the basis of this hypothesis, an explanation may be offered for the constant mitotic activity observed in these experimental tumors. The component of the normal cell, affected by the influences leading to function of the organ of which that cell is a part, in the tumor cell has been partially or completely altered or lost by dedifferentiation. There is thus no, or no regular, shifting of cell activity from the vegetative to the functional. The tumor cell behaves at all times as the normal cell does only during the period of least functional activity. Therefore, the tumor exhibits constant mitotic activity.

#### SUMMARY

The rate and the periodicity of mitotic activity were studied in well differentiated squamous cell carcinoma produced experimentally in male CBA mice by the application of a 0.3 per cent solution of methyl-cholanthrene in benzene. Normal epidermis of the same mice served as the control.

Mitotic activity in the cancers showed no diurnal rhythm but remained at a practically constant level throughout the day and the night. Mitotic activity in the normal epidermis exhibited the characteristic diurnal rhythm.

The rate of mitotic activity in carcinoma was no greater than the maximum rate in an equal volume of normal epidermis.

## TUBERCULOID REACTION IN OVARIAN DYSGERMINOMA

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Dysgerminoma of the ovary is a relatively uncommon tumor. The reported incidence varies from 3.1 per cent (Klaften<sup>1</sup>) to 6.2 per cent (Sailer<sup>2</sup>) of all primary ovarian cancers. The term "disgerminoma" was introduced by Robert Meyer.<sup>3</sup> The inconsistency in the spelling, with the prefixes "dys" and "dis" used with almost equal frequency, is confusing. The prefix "dys" seems preferable, implying "deranged"; thus "dysgerminoma" would literally indicate a growth of deranged germinal cells, which is in accordance with the views of Meyer, whose theory as to the origin of the tumor from undifferentiated embryonic sex cells is rather generally accepted. The tumor has been known by several different names, the more common being "large cell carcinoma of the ovary" and "seminoma ovarii." The testicular counterpart is the seminoma testis (embryonic carcinoma), which histologically bears a striking resemblance to the ovarian dysgerminoma. The clinicopathologic features of the latter have been well described by Novak<sup>4</sup> and Seegar.<sup>5</sup>

Histologically, the dysgerminoma is one of the most constant and characteristic of ovarian tumors. It is composed of compact, fairly small, uniform islands of tumor cells surrounded by a loosely constructed fibrous stroma, giving it a characteristic reticulated structural pattern (*A* in figure). In areas, however, the tumor may grow as broad amorphous masses or in elongated, infiltrative columns; this is especially true of the secondary growths. The tumor cells are large, round and undifferentiated, containing large spherical vesicular nuclei that are often hyperchromatic. The cytoplasm is relatively sparse, stains poorly and is often hydropic. Within the fibrous network sur-

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From the Butler County Memorial Hospital, Butler, Pa.

1. Klaften, E.: Arch. f. Gynäk. **158**:544, 1934.
2. Sailer, S.: Am. J. Cancer **38**:473, 1940.
3. Meyer, R.: Am. J. Obst. & Gynec. **22**:697, 1931.
4. Novak, E.: Gynecological and Obstetrical Pathology, ed. 1, Philadelphia, W. B. Saunders Company, 1940, p. 336.
5. Seegar, G. E.: Arch. Surg. **37**:697, 1938.

rounding the tumor islands are varying numbers of lymphoid cells and at times large irregular cells resembling mononuclear phagocytes of the epithelioid type (*B* in figure). Multinucleated giant cell forms are occasionally encountered.

Interpretations of the nature of the stromal reaction have been somewhat controversial. Novak and Gray<sup>6</sup> considered the epithelioid and giant cells as degenerated tumor cells. To Schiller<sup>7</sup> they are not constituents of the tumor but a product of the stromal reaction. He postulated that the tumor produces a lipid substance similar to that of the tubercle bacillus, which stimulates a tuberculoid stromal reaction. He demonstrated lipoids in the tumor, a finding which has been confirmed by Greenblatt and Pund.<sup>8</sup> Schiller's interpretation would be completely substantiated if the lipid could be shown by chemical and biologic reactions to be related to the lipid of the tubercle bacillus (Sabin, Doan and Forkner<sup>9</sup>).

The origin of the lymphoid cells is not clear, although they are generally considered to be lymphocytic. Seegar<sup>5</sup> suggested that they might be "extruded polar bodies."

Occasionally the stromal reaction in dysgerminoma has assumed a tubercle-like arrangement, actually mistaken for tuberculosis. Kermauner<sup>10</sup> reported five granulosa cell tumors accompanied by tuberculosis, which Schiller<sup>7</sup> in a subsequent review reclassified as dysgerminoma. Schiller concluded that the stromal reaction was not tuberculous.

Only a few isolated cases exhibiting the tuberculoid reaction have been reported in the English literature (Sailer<sup>2</sup>—cases 1 and 2; Greenblatt and Pund<sup>8</sup>—photomicrograph in case 5; Novak and Gray<sup>6</sup>—case 12<sup>11</sup>). At times a granulomatous reaction of a foreign body type has been observed.<sup>12</sup> The 2 cases reported here demonstrate the tuberculoid reaction of dysgerminoma. In case 1 the diagnosis of tuberculous salpingitis complicating the dysgerminoma was seriously considered for

6. Novak, E., and Gray, L. A.: *Am. J. Obst. & Gynec.* **35**:925, 1938.

7. Schiller, W.: *Arch. f. Gynäk.* **156**:513, 1934.

8. Greenblatt, R. B., and Pund, E. R.: *Am. J. Obst. & Gynec.* **35**:667, 1938.

9. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: *Am. Rev. Tuberc.* **21**: 290, 1930.

10. Kermauner, cited by Schiller.<sup>7</sup>

11. The findings in this case were stated to have only a superficial resemblance to tuberculosis.

12. Dockerty, M. B., and MacCarty, W. C.: *Am. J. Obst. & Gynec.* **37**:878, 1939.

a time. Subsequent studies failed to demonstrate the organism. Twelve days later case 2, presenting a similar complicating picture, strengthened the suspicion that the tuberculoid reaction was secondary to the tumor.

#### REPORT OF CASES

CASE 1.—A 22 year old white woman, married, was admitted to the Butler County Memorial Hospital Jan. 7, 1942, complaining of intermittent abdominal pain of three months' duration, which radiated to the right hip and thigh. The previous medical history was irrelevant. The menses had been normal except for the preceding three periods, which were scanty.

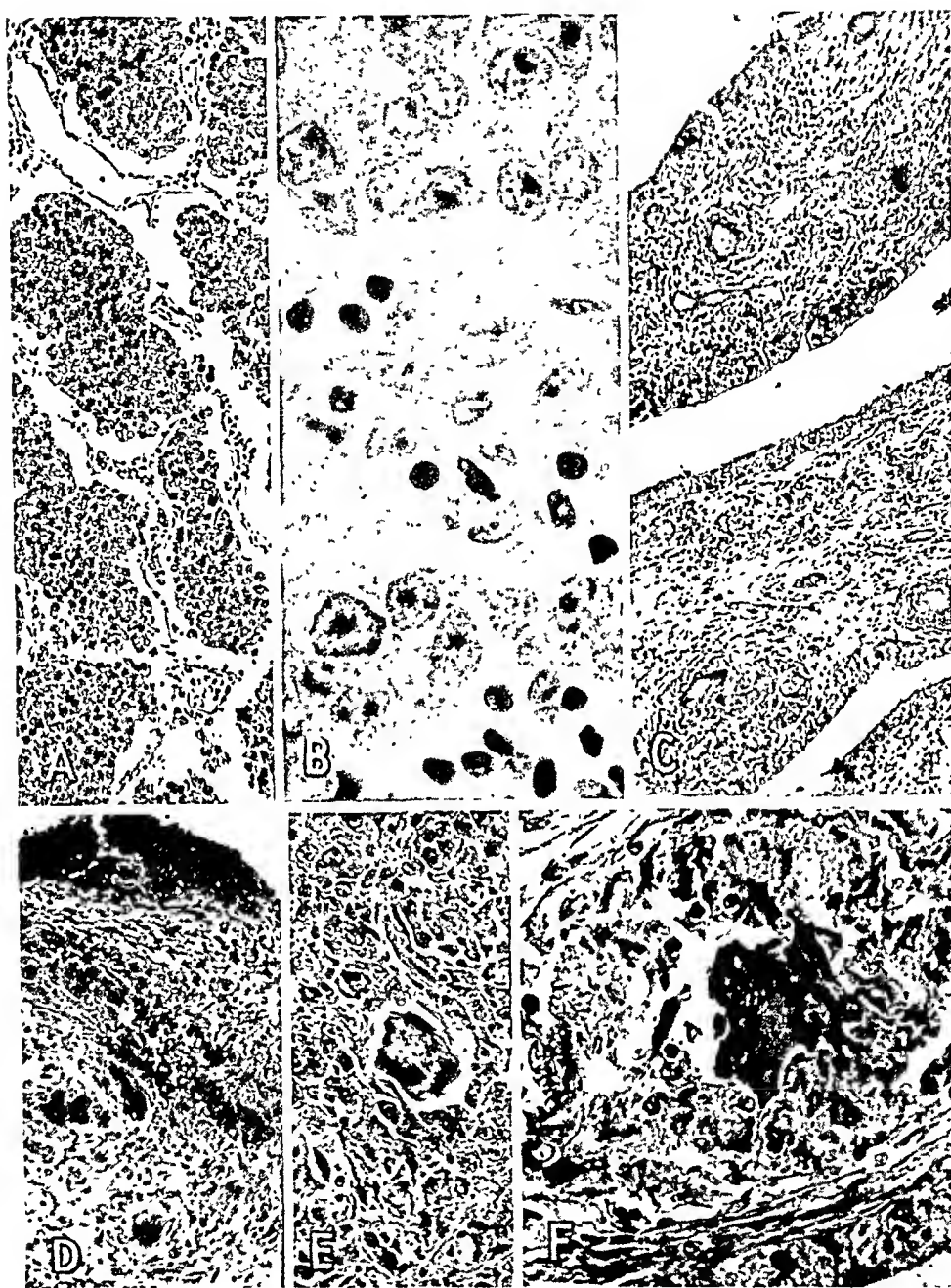
There was a large firm rounded mass in the lower portion of the abdomen. No additional abnormalities were noted.

At operation (Dr. C. A. Robb) the mass completely replaced the right ovary and had extensively infiltrated the right fallopian tube and broad ligament; metastases were noted in many of the regional and paravertebral lymph nodes and the opposite ovary. The adnexae uteri on both sides and the tumor mass were resected with difficulty.

The postoperative course was uneventful except for several days of intermittent fever. There were no signs of pulmonary disease on roentgen examination after operation. A course of treatment with high voltage roentgen rays was given postoperatively, during which the palpable tumor nodules rapidly regressed until they were no longer detectable. At the time of writing, approximately one year after operation, the patient presents no evidence of recurrence and is free of complaints.

*Operative Specimen.*—This consisted of a large rounded tumor mass, an ovary and a thickened, distorted broad ligament. The tumor mass measured 14 by 11 by 8 cm. Its surface was irregularly nodular and was covered with a thin fibrous capsule, which in areas was ragged and hemorrhagic. The tumor tissue was light yellowish gray and moderately firm. On section it was irregularly and coarsely lobulated by fibrous septums; the tumor tissue was compact, glistening and translucent. A few scattered small areas of softening and degeneration were noted. Along one edge of the mass was a distorted fallopian tube, the wall of which was irregularly nodular and thickened by infiltrating tumor. The opposite ovary was of normal size; protruding from its capsule at one point was a flattened pea size yellowish pink papular nodule which extended but a short distance into the underlying stroma. It was solid and resembled tumor. The broad ligament was diffusely infiltrated by tumor.

Microscopically, the tumor was a typical dysgerminoma. The stromal reaction was pronounced in certain areas. The outermost portion of the fallopian tube was extensively infiltrated with tumor which at times extended to the base of the plicae; rarely the tumor infiltrated the stroma of the plicae. Associated with the tumor, but often quite remote from it, was a granulomatous inflammatory reaction with formation of numerous miliary lesions strikingly similar to tubercles (*C* in figure). These were frequently situated within plicae uninvolved by tumor. Many such lesions were noted in the intimal layer of larger veins at some distance from the tumor (*D* in figure). The small nodule in the left ovary consisted of infiltrative columns of dysgerminoma cells associated with a pronounced tuberculoid reaction of the stroma.



EXPLANATION OF PLATE

*A*, typical structural pattern of dysgerminoma in case 1.  $\times 120$ .

*B*, epithelioid and lymphocytic infiltration of the stroma between islands of tumor cells in case 1.  $\times 700$ .

*C*, pseudotubercles in the plicae of a fallopian tube in case 1.  $\times 76$ .

*D*, polypoid granulomatous reaction of the intima of a vein in the wall of the involved fallopian tube in case 1. The tumor has not involved the vessel.  $\times 185$ .

*E*, dysgerminoma with pronounced stromal reaction in case 2.  $\times 153$ .

*F*, pseudotubercle of the fallopian tube in case 1.  $\times 375$ .



CASE 2.—A 25 year old white woman, married, entered Butler County Memorial Hospital Jan. 19, 1942, complaining of pain in the lower part of the abdomen of one week's duration. The last menstrual period occurred in the preceding July, and she considered herself to be approximately six months pregnant. She had had no previous pregnancies. The remainder of the history was irrelevant. Physical findings were normal except for a large rounded mass in the lower part of the abdomen, which extended upward to the level of the umbilicus.

At operation (Dr. C. A. Robb) two large solid rounded tumor masses replaced both ovaries. The capsule of the tumor on the left was extensively adherent to the posterior surface of the uterus, the omentum, loops of intestines and the parietal peritoneum. The uterus was of normal size (nulliparous). Neither metastasis nor local infiltration was apparent. The tumor masses, the uterus and both tubes were removed. Convalescence was uneventful except for several days of intermittent fever. Roentgenograms of the chest taken postoperatively revealed normal-appearing heart and lungs. The patient received a course of treatment with high voltage roentgen rays postoperatively. At the time of writing, approximately one year later, there is no evidence of recurrence, and the patient has no complaints.

*Operative Specimen.*—This consisted of two large tumor masses, the uterus and torn portions of the fallopian tubes. The tumors were approximately of equal size, measuring individually 16 by 12 by 7 cm. Their combined weight was 1,370 Gm. They were covered by thin fibrous capsules; in areas they were hemorrhagic and covered by adhesions. The masses were moderately firm, yellowish gray and nodular. On section the cut surface bulged slightly, was compact and glistening and showed areas of hemorrhage and necrosis. Various sized lobules were formed by delicate fibrous septums.

The uterus was small, with its serosa thickened and covered with torn adhesions and portions of the adnexae.

The tubes were slightly dilated and distorted by adhesions.

Microscopically, the tumor was typical dysgerminoma, with a pronounced stromal reaction, containing lymphocytes, epithelioid cells and scattered giant cells (*E* in figure). The giant cells were frequently of the Langhans type; rarely tubercle-like nodules were observed. There was no extension of the tumor to the fallopian tubes or to the uterus. The sections of tubes revealed moderate infiltration by chronic inflammatory cells but were in no manner suggestive of tuberculosis.

#### COMMENT

The stromal reaction in the 2 cases reported was unusual and pronounced although fundamentally similar to that of typical dysgerminoma. There was an unusually pronounced epithelioid cell infiltration, accompanied by a profuse lymphoid reaction. The tuberculoid reaction in case 2 resembled tuberculous granulation tissue without tubercles and was so intimately associated with the tumor that it would scarcely be misinterpreted as tuberculous. The tuberculoid reaction was more pronounced in the infiltrative secondary lesions of case 1 and at times occurred in the advancing borders, not intimately associated with the tumor cells.

Consideration of the following points has led to the conclusion that the epithelioid and the giant cells were inflammatory rather than neoplastic.

*Morphology* (phloxine-methylene blue stain).—The epithelioid cells showed considerable variation in size and contour in contrast with the tumor cells, which were notably uniform in size and shape. The relative proportion of cytoplasm to nucleus was much greater in the epithelioid than in the tumor cells. In the epithelioid cells, the cytoplasm was constantly acidophilic, and the nuclei were hypochromatic and vesicular. In the tumor cells, the cytoplasm was neutrophilic or lightly basophilic, and the nuclei were larger and made up of coarser chromatin masses.

The giant cells in these 2 cases were distinctive and characteristic, often containing a dozen or more nuclei. The cytoplasm was particularly abundant and retained the acidophilic staining reaction of the individual epithelioid cells. Morphologically, the giant cells were often indistinguishable from the Langhans giant cells of tuberculosis (*F* in figure). Epithelioid cells in the process of fusion could be seen frequently. In contrast, the tumor cells sometimes contained two or more nuclei, and apparent fusion with adjacent cells was noted, but they never formed giant cells of the Langhans type. Occasionally, discrete tumor cells were seen in the stroma adjacent to the epithelioid cells, from which they could be readily differentiated.

*Mitotic Figures*.—An exhaustive oil immersion study failed to reveal a single mitotic figure in the epithelioid cells, although large numbers of mitotic figures were present in the tumor cells.

*Associated Cells*.—Lymphoid cells within the stroma in previously reported cases of dysgerminoma were generally interpreted as true lymphocytes. In these 2 cases the lymphoid cells were numerous and were accompanied in smaller numbers by plasma cells and eosinophils. The latter two cell types are known to be inflammatory, and their presence strengthens the belief that the other stromal elements are a part of an inflammatory reaction. The presence of plasma cells and occasional eosinophils was previously observed by Spielman and Morton.<sup>13</sup>

*Tubercle Formation*.—The pseudotubercles with epithelioid and giant cells, occurring in the stroma, were usually associated with peripheral fibrosis. This often occurred at some distance from the tumor. In the cellular islands of neoplastic tissue, where connective tissue was scanty or absent, pseudotubercles were not found. They were present in the intima of veins at some distance from the tumor, which was interpreted as evidence that they were small foreign body granulomas stimulated by

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13. Spielman, F., and Morton, F. L.: *Am. J. Obst. & Gynec.* **36**:665, 1938.

some product of the tumor. The presence of a lipoid substance in dysgerminoma suggested that this substance might be the foreign body about which the reaction occurred. The interpretation of Schiller seems to be the best explanation for the presence of pseudotubercles in dysgerminoma.

#### SUMMARY

Two cases of ovarian dysgerminoma accompanied by an unusual tuberculoid stromal reaction are reported. The epithelioid and giant cells are most likely inflammatory in character rather than neoplastic.

# A POSTMORTEM STUDY OF THE RENAL PELVIS IN RELATION TO HYPERTENSION

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AND

LEWIS L. KLINE, M.D.

DETROIT

Interest has recently been aroused in the relation between the anatomy of the renal pelvis and hypertension. Campbell<sup>1</sup> in 1941 reviewed 137 cases of infravesicular obstruction, in 19 of which the obstruction was associated with hypertension. He suggested that hypertension should occur more readily in those cases in which the pelvis is in the intrarenal position.

Ravich<sup>2</sup> went further, saying that in the human species a minority retain throughout life a fetal type of kidney on one or both sides, in which the pelvis is in the intrarenal position. He stated further that the intrarenal pelvis tends to persist in certain families throughout life and that in this group hypertension frequently develops. He explained that the mechanism consists of two factors; the first factor is a pelvis in the intrarenal position, and the second, a back pressure developing in the pelvis because of some superimposed condition. The distended pelvis in a narrow hilus was thought to exert pressure on the large renal arteries, producing renal ischemia and thereby hypertension. However, if the same conditions obtain in a kidney with the pelvis in the extrarenal position, on account of the wide hilus a major portion of the pelvis is not closely surrounded by renal parenchyma, and severe dilatation can occur without appreciable pressure on the renal vessels. Ravich supported this theory by a review of 200 intravenous urograms. He was "amazed at discovering the regularity with which hypertension was accompanied by disease in the intrarenal type of kidney pelvis. On the other hand, because of the cushioning effect inherent in the extrarenal type, essential hypertension in such protected kidneys appeared extremely unlikely even though the existing pathologic changes were of considerable degree."

More recently Sarnoff<sup>3</sup> examined the pyelograms in a series of cases of hypertension and concluded that there was no significant relation

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1. Campbell, E. W.: *J. Urol.* **45**:70, 1941.

2. Ravich, A.: *J. Urol.* **46**:641, 1941.

3. Sarnoff, S. J.: *J. Urol.* **47**:769, 1942.

between the type of renal pelvis and hypertension as judged by clinical urography.

The purpose of our study was to determine by the use of postmortem urography and statistical analysis whether there is any significant correlation between the position of the renal pelvis and hypertension.

#### METHOD AND MATERIAL

A radiopaque mass was injected in a retrograde manner into the ureter and renal pelvis under controlled pressure. A permanent record was obtained by making a roentgenogram of the injected specimen. The injection mass and the apparatus were similar to those used by Schlesinger<sup>4</sup> for the study of hearts.

With a wax pencil, the hilar rim was then outlined on the roentgenogram by comparison with the gross specimen. Actual measurement was employed when the hilar rim was not plainly discernible on the roentgen plate. Sections were then taken for microscopic study.

In this manner it was possible to record exactly the position of the renal pelvis, the hilar rim, the contour and the size of the kidney, the proximity of the parenchyma to the pelvis and the calices, or other anatomic features of the specimen.

The series consisted of 76 cases which were, in the main, consecutive. No selection was attempted, and the group is representative of cases which come to necropsy in the hospital.

By measurement of the pyelogram it is possible to locate exactly the pelvis in relation to the parenchyma of the kidney. This was done in the following manner: A triangle was drawn between the ureteropelvic junction and the bottom portion of the superior and that of the inferior major calix. The center of such a triangle was found at the intersection of two lines drawn, one from each base angle to the midpoint of the opposite side. This point, while not the true center of the pelvis, serves as a point of reference by which the type of pelvis in any individual specimen can be determined.

This method being used, an intrarenal pelvis is defined as a pelvis in which the reference point falls lateral to or within the hilar rim (*A* in figure). By definition an extrarenal pelvis is one in which the point of reference falls medial to the hilar rim (*B* in figure).

Cases in which the diastolic pressure was 90 mm. or above or in which the microscopic sections of the kidney revealed arteriosclerosis were considered to show hypertension. The series was then arranged in four groups depending on the position of the pelvis and on the presence or absence of hypertension. The results of this grouping are seen in the accompanying table.

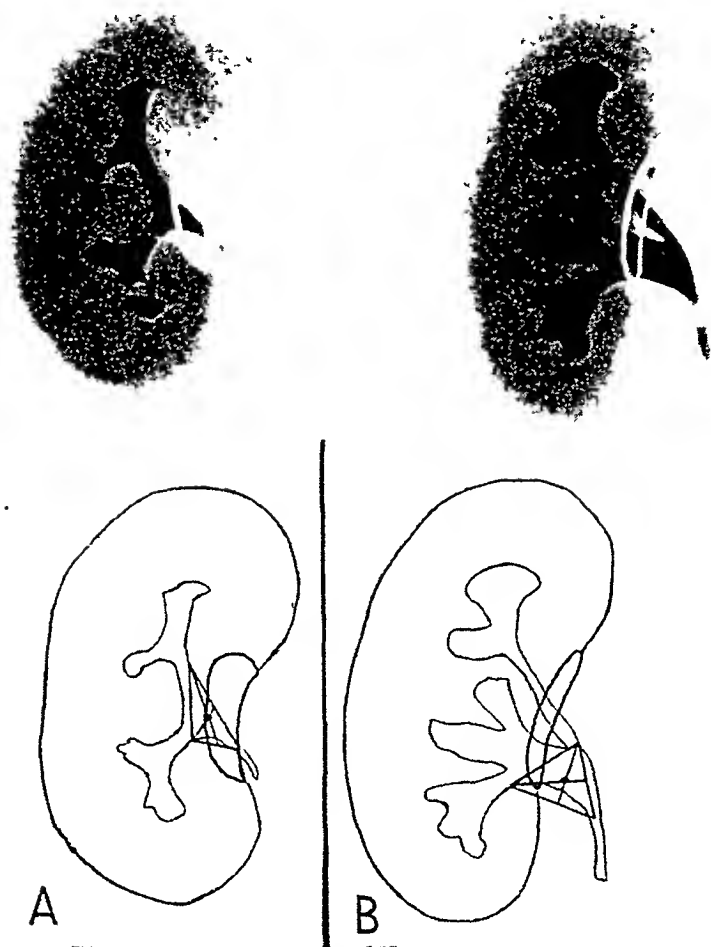
It has been demonstrated by several investigators<sup>5</sup> that ischemia of only one kidney causes hypertension. Since such is the case, it seemed only fair that for the purpose of mathematical analysis all cases in which one kidney was of the extrarenal and the opposite of the intrarenal type should be included in the intrarenal group. In the table 14 such cases are placed in the intrarenal division. These account for 18.4 per cent of the total 76 pairs of kidneys.

A survey of our tabulated data does not lend support to the theory proposed by Ravich. On inspection of the table it may be seen that the pelvic types were equally represented, there being 38 cases of each.

4. Schlesinger, M. J.: *Am. Heart J.* **15**:528, 1938.

5. Goldblatt, H.: *Am. J. Clin. Path.* **10**:40, 1940. Wilson, C., and Byrom, F. B.: *Lancet* **1**:136, 1939. Page, I. H.: *J. A. M. A.* **113**:2046, 1939.

The cases in which hypertension was present were about equally divided, 14 in the intrarenal and 15 in the extrarenal group. These 29 cases in which hypertension was present make up about 38 per cent of the total number of cases studied. Likewise, the cases in which no hypertension was observed were fairly equally distributed between the two aforementioned groups. When these data were subjected to the chi square test, the value for chi square was found to be 0.0552 and the



Roentgenograms of kidneys, with tracings to illustrate the location of the reference point in a case of intrarenal pelvis (A) and in a case of extrarenal pelvis (B).

corresponding probability 0.8132. This supports the contention that there is no relation between the anatomic type of pelvis and the development of hypertension because with a probability value of 0.8132 there is an eight to ten chance that the slight difference in number of cases in each group is due to errors in sampling alone.

The fact that kidneys as found in the human species are frequently rotated on the sagittal axis so that the pelves are directed anteriorly or

posteriorly was exhaustively investigated by Kelly and Burnam.<sup>6</sup> These investigators found that the topography of the renal vessels was greatly affected by the degree of rotation. In the more severe degrees of rotation the majority of the vessels tend to follow a curved course over the hilar rim before entering the parenchyma. It seemed theoretically possible that ischemia might be more readily produced when pressure is exerted on the severely angulated vessels, bordered as they are on one side by unyielding renal substance.

Our series was regrouped and statistically analyzed for the relation between rotation and hypertension. No significant degree of correlation was found. The fact that fetal lobulation was frequently associated with the immature type of kidney has been mentioned.<sup>2</sup> We therefore studied in a like manner the relation between fetal lobulation and hypertension and could demonstrate no significant correlation.

*Grouping of Cases in Series\**

Type of Pelvis	Hyper- tension	No Hyper- tension	Total
One or both intrarenal.....	14	24	38
Both extrarenal .....	15	23	38
Total.....	29	47	76
Chi square = 0.0552      Probability = 0.8132			

\* Pearl, R.: *Medical Biometrics and Statistics*, ed. 3, Philadelphia, W. B. Saunders Company, 1940.

During the course of this investigation it became obvious that rotation of the kidney makes intravenous urography an unreliable method of determining the type of renal pelvis.

We therefore conclude that in this series there is no statistical evidence to support the contention that hypertension and intrarenal position of the pelvis are related. Nor do we feel that the degree of rotation or fetal lobulation is related to hypertension. It is interesting to note that the conclusions drawn by Sarnoff,<sup>3</sup> based on antemortem pyelography, agree essentially with the conclusions at which we arrived by the study of postmortem urographs.

SUMMARY

A method for exactly determining the position of the pelvis relative to the parenchyma of the kidney is described. This method was applied in a representative series of routine autopsies. The results were statistically analyzed to determine any significant correlation between the intrarenal pelvis and hypertension. No such correlation could be demonstrated. Likewise, no significant correlation was found between hypertension and rotation or between hypertension and fetal lobulation.

6. Kelly, H. A., and Burnam, C. F.: *Diseases of the Kidneys, Ureters and Bladder*, with Special Reference to the Diseases in Women, New York, D. Appleton and Company, 1914; ed. 2, 1922.

# TYROSINE POISONING IN RATS

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AND

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Tyrosine occupies a key position in the production of epinephrine, melanin, thyroxin and posterior pituitary hormone. As a precursor of tyramine it has been related to the causation of "pale hypertension," while as the chief component of melanin it has been suspected as a factor responsible for the incidence of primary cancer of the liver among the colored races. Umber,<sup>1</sup> as well as Weiss,<sup>2</sup> commented on the urinary excretion of tyrosine in hepatic diseases and its relation to the frequently coexisting pancreatitis, whereas Baer and Blum<sup>3</sup> and Neubauer<sup>4</sup> stated that tyrosine is one of the four amino acids (leucine, isoleucine, tyrosine and phenylalanine) responsible for the formation of ketone bodies. Bickel<sup>5</sup> noted the aglycogenoplastic properties of tyrosine, as it prevents the accumulation of glycogen in the liver, and called attention to the importance of this amino acid in the dietary management of diabetes.

Litzka<sup>6</sup> mentioned that tyrosine fluoride causes a reduction of the blood sugar in patients with diabetes or exophthalmic goiter, and Abelin<sup>7</sup> recommended, on the basis of animal experiments, the administration of tyrosine to patients with hyperthyroidism as a detoxicant of the thyroid hormone. A similar suggestion was made by Medes,<sup>8</sup> who warned, however, against the use of excessive doses of this substance, as she observed directly following such medication, an increase in basal metabolism, an effect which was also noted by Abelin.

Concerning the possible toxic effects of such a treatment, Abelin stated that tyrosine is rapidly metabolized in the animal organism and

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From the Warner Institute for Therapeutic Research.

1. Umber, F.: *Erkrankungen der Leber, der Gallenwege und des Pankreas*, in Mohr, L., and Staehelin, R.: *Handbuch der inneren Medizin*, Berlin, Julius Springer, 1926, vol. 3, pt. 2, p. 1.

2. Weiss, M.: *Klin. Wchnschr.* **15**:521, 1936.

3. Baer, J., and Blum, L.: *Arch. f. exper. Path. u. Pharmakol.* **56**:92, 1907.

4. Neubauer, O.: *Intermediärer Eiweißstoffwechsel*, in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: *Handbuch der normalen und pathologischen Physiologie*, Berlin, Julius Springer, 1928, vol. 5, p. 851.

5. Bickel: *Klin. Wchnschr.* **18**:178, 1939.

6. Litzka, G.: *Klin. Wchnschr.* **15**:1568, 1936.

7. Abelin, I.: *Klin. Wchnschr.* **14**:1777, 1935.

8. Medes, G.: *Biochem. J.* **26**:917, 1932.



can be given in large amounts without any danger. This claim is apparently confirmed by the observations of Abderhalden and co-workers,<sup>9</sup> who took within one day from 50 to 150 Gm. of tyrosine without any apparent ill effect. Similarly, Medes administered to normal persons from 5 to 10 Gm. and occasionally as much as 50 Gm. of this amino acid without their experiencing untoward reactions. Newburgh and Marsh,<sup>10</sup> on the other hand, when working with dogs and rabbits noted that tyrosine given in doses of 2 Gm. per kilogram of body weight causes renal injury. Lillie<sup>11</sup> recorded the occurrence of minor tubular degenerations of the kidney, exudative blepharitis and red, edematous swelling of the extremities in rats kept for as long as twenty days on a basal diet containing as much as 20 per cent tyrosine (Sullivan, Hess and Sebrell<sup>12</sup>). Liver, small intestine, spleen, skeletal muscle and eye, also studied, were without abnormalities.

The observations to be reported were made during investigations on the role of tyrosine in the causation of hypertension, conducted by one of us (Martin<sup>13</sup>), and represent a continuation of previous experimental studies in cardiovascular pathology (Hueper<sup>14</sup>).

#### EXPERIMENTAL OBSERVATIONS

Black rats, 5 weeks old, were placed on a basal diet consisting of sucrose 57 parts, casein 18 parts, cod liver oil 2 parts, butter fat 9 parts and salts 4 parts, with the addition of thiamine hydrochloride 5 mg., riboflavin 10 mg., pyridoxine 5 mg., calcium pantothenate 100 mg., inositol 100 mg., paraaminobenzoic acid 100 mg., nicotinic acid 100 mg. and choline 200 mg., and the replacement of 10 additional parts of sucrose usually present in the diet by an equivalent amount of l-tyrosine (Martin). The l-tyrosine was supplied by Dr. Arnold H. Johnson, Research Laboratories, Sealtest, Inc., Baltimore. With the tyrosine contained in the casein, the diet thus contained 11.17 parts of tyrosine. Within one week the animals had crusted eyelids, opacities and ulcers of the cornea and swollen, red legs and feet. The majority of the rats died within three weeks, while a few survived almost five weeks (thirty-three days). Of the large number of rats thus treated and used for measurements of blood pressure and chemical studies of the blood, 83 were available for pathologic investigations. Forty-two of these rats died spontaneously, and 41 were killed for the purposes just mentioned. Twenty-five rats survived for seven to ten days, 50 for eleven to nineteen days and 8 for twenty to thirty-three days.

The autopsy showed the meningeal vessels engorged and, in some animals, surrounded by large hemorrhagic areas. The lungs were congested and frequently

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9. Abderhalden, E.: *Ztschr. f. physiol. Chem.* **77**:454, 1912

10. Newburgh, L. H., and Marsh, P. L.: *Arch. Int. Med.* **36**:682, 1925.

11. Lillie, R. D.: *Pub. Health Rep.* **47**:83, 1932.

12. Sullivan, M. X.; Hess, W. C., and Sebrell, W. H.: *Pub. Health Rep.* **47**:75, 1932.

13. Martin, G. J.: *Arch. Biochem.*, to be published.

14. Hueper, W. C.: *Arch. Path.* **34**:883, 1942.

contained hemorrhagic foci. The heart was contracted. The pancreatic tissue was embedded in an edematous matrix in an appreciable number of animals and was enlarged in mass in others. The liver and the spleen were grossly normal. The kidneys of a considerable number of rats showed a finely granular, light brownish surface. The testes were small, corresponding to the age of the animals.

The histologic examination of the various organs rendered the following results:

The meningeal vessels were often highly congested and not infrequently surrounded by extensive hemorrhagic areas. In some instances there existed considerable perivascular fibrosis. Similar changes were present along the vessels extending into the brain substance. The cerebral vessels were usually hyperemic also and often surrounded by small hemorrhages. The arterioles were contracted and exhibited, particularly in rats which survived for more than ten days, swelling and hyalinization of the vascular walls (fig. 1*A*). Some of the vessels showing these changes were surrounded by glia cells and leukocytes; others, by an edematous zone. The brains of a few rats contained small necrotic foci with glial and leukocytic infiltrations. The ganglion cells not infrequently exhibited degenerative changes, such as balloon-like swelling and vacuolation, while in a rat which survived for twenty days a group of calcified ganglion cells was observed (fig. 1*B*).

The anterior lobe of the hypophysis was congested in most instances and was composed of strands of eosinophilic cells surrounding groups of chromophobic cells. The median and posterior lobes were normal.

Many of the eyeballs examined showed mild to severe degenerative and inflammatory changes of the cornea. These extended rarely to the eyelids. In the mild conditions the corneal lesions consisted of edema and scanty infiltration by mononuclear cells and leukocytes and were most marked in the marginal portions. In more advanced conditions, involving the entire cornea, there was dense accumulation of leukocytes and lymphocytes, associated with invasion of capillary vessels. The epithelial lining was eroded, and the ulcerative defects were covered by a leukocytic exudate. The rest of the eyeball was intact.

The thyroid glands of a few animals were normal. The glands of the majority and particularly those of rats surviving for more than twenty days consisted of small follicles filled with thin, vacuolated colloid. There were, moreover, areas of solid follicles and follicles with a proliferated epithelial lining.

The lungs were usually congested, and many of them contained scattered hemorrhagic areas. The pulmonary arterioles of 8 rats exhibited thickened and often hyaline walls lined by a crowded endothelium arranged in palisade formation.

A small myocardial hemorrhage was found in a single rat, while a small focus of myocardial calcification was present in a second. The myocardial arterioles were thickened in 10 animals and were hyaline in some. The frequency of this change increased with the duration of the treatment.

The liver often showed some degree of congestion. The liver cells had in many instances a finely granular cytoplasm, while in some the liver exhibited a moderate degree of pericentral fatty degeneration with large vacuoles or contained scattered small hyaline necroses.

The spleen was in general normal. In a few instances there was general atrophy of the pulp, associated with scattered hyaline necroses.

The stomach and intestines showed no abnormality.

The pancreas was normal in 17 instances. In all others it showed more or less extensive lesions. The interstitial connective tissue was often highly edematous



Fig. 1.—*A*, meningeal arterioles with polypoid endothelial proliferations. *B*, small focus of necrosis in the cortex, with accumulation of glia cells, and pyknosis and calcification of ganglion cells. *C*, edema and fibroblastic proliferation in the interstitial tissue of the pancreas, with degeneration and necrosis of some pancreatic lobules.

and infiltrated to varying degrees by large mononuclear cells and eosinophilic leukocytes (fig. 1 *C*). The pancreatic lobules which were embedded in this matrix sometimes contained only a few necrotic foci, usually infiltrated and surrounded by eosinophilic leukocytes. In other instances there was evidence of a prolonged and progressive degenerative process. The exocrine cells of some irregularly shaped acini were small and free from zymogen granules. They possessed a faintly stained, vacuolated cytoplasm and indistinct outlines. Their nuclei were pale, irregular in shape and reduced in size. In other lobules this process had progressed further. The acini were necrotic or had collapsed after the lysis of the cells, leaving small cysts. The increased intralobular connective tissue frequently formed nodules of large spindle-shaped cells, infiltrated by lymphocytes and eosinophilic leukocytes (fig. 2 *A*). These were also found in many instances within the necrotic exocrine glandular foci. The islets were usually normal in appearance and number during this stage, or they showed moderate to marked swelling, characterized by the appearance of strands of large vesicular cells in loose gyriform formations. In still older lesions much of the pancreatic tissue had been replaced by hyaline necroses and fibroblastic tissue containing usually a varying number of mononuclear cells and eosinophilic leukocytes. The general pattern was that of a multinodular granuloma, throughout which small remnants of the pancreatic tissue were scattered. There was marked reduction in the number of islets, which had been engulfed in the general cirrhosis (fig. 2 *B*). However, isolated islets surrounded by large areas of fibrous tissue were found occasionally. The blood vessels and the ducts were in general normal. Moderately dilated ducts occurred only infrequently.

In the adrenal glands, the medullary sinuses were often engorged. A diffuse hemorrhage into the medulla and the cortex was present in 1 case.

The kidneys in the majority of instances showed hyaline nephrotic changes. In approximately 30 per cent of the cases they showed, moreover, focal necrotizing lesions, involving the cortex as well as the medulla, often affecting wedge-shaped zones. The mildest reactions of this type consisted in accumulations of leukocytes in the tubular lumens, with moderate leukocytic infiltration in the surrounding tissue, and cystic distention of tubules, with atrophic, metachromatic, dark-stained cells in the corresponding cortical areas. The cystically distended tubules contained occasionally an albuminous pink or brown matter and were surrounded by thickened and cellular interstitial tissue. In more advanced lesions there were central hyaline necroses surrounded by leukocytes and fibroblasts. The pink-stained necrotic centers consisted of a peculiarly filamentary matter arranged in radiating fashion (fig. 2 *C*). In several instances there was diffuse granulomatous involvement of the tip portion of the pyramids. The pelvic epithelial lining was then eroded and covered by thick leukocytic exudate which also filled the renal pelvis. The glomeruli were usually intact. The arterioles in an appreciable percentage showed thickened and sometimes hyaline walls and occasionally proliferation of the endothelial cells.

In the testes, the spermatogenic epithelium was in general of the immature type found normally in animals of this age. However, in 8 rats which had been exposed for more than ten days to the tyrosine diet, definite evidence of epithelial atrophy and of spermatid giant cell formation was obtained.

The marrow of the sternum was frequently highly congested and in an appreciable number of instances contained large hemorrhages. The latter condition was associated in general with moderate to marked atrophy of the myeloid tissue. A few additional animals exhibited atrophic myeloid tissue with scattered hyaline necroses.

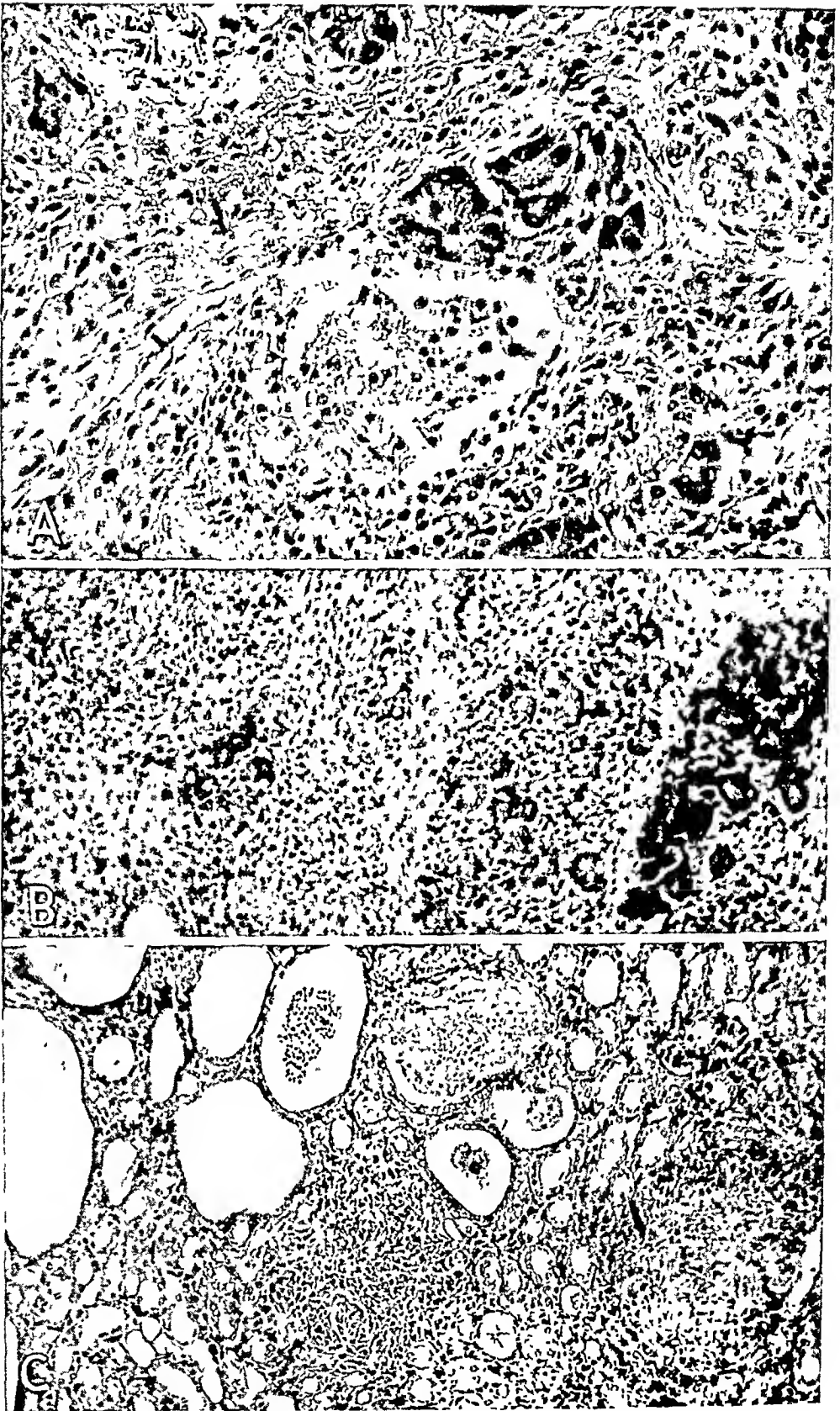


Figure 2

*(See legend on opposite page)*

## COMMENT

The recorded observations, some of which confirm those previously reported by Lillie,<sup>11</sup> reveal that tyrosine poisoning as produced in rats is characterized by two main types of pathologic changes, those of degenerative and necrotizing nature, affecting the parenchyma of the pancreas, the eye, the kidney, the brain, the liver, the spleen, the testes and the marrow, and those of a circulatory-vascular genesis, represented by congestion, hemorrhages, edema, perivascular fibrosis and arteriolar medial degeneration, affecting the brain, the lungs, the heart, the adrenal glands, the kidneys and the marrow.

The most striking and extensive inflammatory, degenerative and cirrhotic lesions were found in the pancreas. The intact status of the pancreatic vessels, as well as the primary involvement of the exocrine tissues of this organ, does not favor the possibility that these lesions are the result of spastic arteriolar ischemia of the pancreatic vessels elicited either by intrapancreatic transformation of tyrosine into tryamine or by excessive production of epinephrine under the influence of an abnormal supply of tyrosine (Holtz<sup>15</sup>). It is also not likely that these changes were caused by a specific antipancreatic action of the secretion of the anterior lobe of the pituitary gland, possibly stimulated by tyrosine, since the degenerative changes produced in the pancreas by prolonged treatment with anterior pituitary extract are restricted to the islets and cannot be elicited in rats, though elicited readily in dogs and less easily in cats and rabbits (Young<sup>16</sup>; Marks and Young<sup>17</sup>; Richardson<sup>18</sup>). It is even maintained that the repeated introduction of anterior pituitary extract into rats is followed by hypertrophy and multiplication of Langerhans' islands (Anselmino, Herold and Hoffmann<sup>19</sup>).

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18. Richardson, K. C.: *Proc. Roy. Soc., London, s.B* **128**:153, 1940.

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EXPLANATION OF FIGURE 2

*A*, nodular fibrosis of the pancreas, with hypertrophy of the islets and interstitial infiltration by eosinophilic cells.

*B*, advanced hyaline fibrosis of the pancreas, with small remnants of exocrine and endocrine pancreatic tissue.

*C*, multiple necrotic foci with cystically distended renal tubules.

The presence of purulent keratitis in many of the rats suggested that tyrosine or one of its metabolites might have caused an imbalance of the vitamin metabolism, as such ocular manifestations are found with various deficiency states due to lack of factors of the vitamin B complex, and as pancreatic atrophy and fibrosis sometimes accompany thiamine avitaminosis (Eddy and Dalldorf<sup>20</sup>; Wolbach<sup>21</sup>; Kihn<sup>22</sup>). However, the absence of any additional signs of thiamine deficiency and the rapid development of the pancreatic lesions in the rats kept on a tyrosine diet militate against the action of an avitaminotic mechanism.

There remains the possibility that the degenerative and necrotizing processes in the pancreas were caused either by activation of the pancreatic proteolytic enzymes by the tyrosine or by direct toxic action of tyrosine, excreted perhaps through the exocrine tissues, on these elements. In considering these possibilities it may be noted that diseases of the biliary system are often associated with tyrosinemia (Umber<sup>1</sup>; Weiss<sup>2</sup>; Jankelson, Segal and Aisner<sup>23</sup>) and that pancreatitis and diabetes occur often as the so-called "second disease" in these conditions (Terbrüggen<sup>24</sup>; Gruber<sup>25</sup>). While it is assumed by many investigators that these pancreatic complications are the result of penetration of bile into the pancreatic duct and the activation of the proteolytic enzymes ensuing therefrom and causing inflammation and destruction of the pancreatic tissue, this contention has not remained unchallenged (Westphal<sup>26</sup>). The present observations suggest that a different and intrapancreatic mechanism connected with the tyrosinemia may be operative. It is obvious that diabetes mellitus developing on the basis of tyrosine poisoning is secondary to pancreatitis.

The degenerative and necrotizing lesions found in the renal parenchyma and pelvis are evidently of an excretory and toxic character and are unrelated to the arteriolar hyaline changes observed in some of the kidneys. The wedge-shaped distribution of the tubular involvement and especially the epithelial erosion at the tips of the pyramids support

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21. Wolbach, S. B.: *Growth and Development of the Child: III. Nutrition*, New York, The Century Company, 1932.

22. Kihn, B.: *Avitaminosen und verwandte Krankheitszustände*, Berlin, Julius Springer, 1927.

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25. Gruber, G. B.: *Pathologie der Bauchspeicheldrüse*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1929, vol. 5, pt. 2, p. 211.

26. Westphal, K.: *München. med. Wchnschr.* **2**:1553, 1936.

this interpretation. A toxic factor is apparently also responsible for the foci of degeneration found in the liver, the spleen and the testes of some of the rats. Circulatory disturbances seem to be, on the other hand, the main cause of the foci of necrosis and degeneration observed in the brain and in the marrow, as these exhibited a close relation to hemorrhages or vascular lesions.

The degenerative medial changes found in an appreciable number of arterioles of the brain, the heart, the lung and the kidneys, sometimes associated with endothelial proliferation, represent the second remarkable feature of tyrosine poisoning, as they were elicited in rats of an age at which spontaneous occurrence of such lesions is unknown. The mechanism active in their production is uncertain, however. It is possible, though not likely, that tyrosine or a metabolite may have exerted a direct toxic effect on the vascular walls. However, inasmuch as the rats studied showed hypertension symptomatically (Martin), it is more probable that a hypertensive mechanism is involved in this respect, as tyrosine may give rise to the vasopressor amine, tyramine, or may be used in the production of epinephrine or of a vasopressor hormone of the pituitary or the thyroid gland. Consideration must be given, moreover, to the possibility that a renal pressor agent may have contributed to any pressor effect, as the kidneys of many rats revealed extensive degenerative tubular and interstitial mononuclear and fibroblastic changes.

The observations made are important for various reasons. They suggest interrelations between disturbances in the tyrosine metabolism, diseases of the biliary tract and the development of pancreatitis and diabetes mellitus. They add to information concerning the relationship between the activity of vasopressor agents and arteriosclerosis and arteriolosclerosis. They represent, finally, a warning against the introduction of excessive amounts of this amino acid for therapeutic purposes, as may occur during the proposed treatment of thyrotoxicosis.

#### SUMMARY

Rats kept on a diet containing 10 per cent 1-tyrosine show within one to two weeks purulent keratitis and swelling and redness of the feet and legs, and die within five weeks.

They show degenerative, necrotizing and fibrosing changes of the pancreas, affecting primarily the exocrine tissue, but leading ultimately to extensive destruction of the exocrine and endocrine tissues.

In the kidneys additional degenerative and necrotizing lesions are found, which apparently have their genesis in the excretion of a toxin.

The arterioles of the brain, the heart, the lungs and the kidneys exhibit swelling and hyalinization of the media and occasionally pro-



liferation of the intima, associated with focal hemorrhages, necroses and glia cell accumulations in the brain.

The observations suggest causal relations between diseases of the biliary tract, pancreatitis, diabetes and disturbances of the tyrosine metabolism and represent a warning against prolonged administration of excessive amounts of tyrosine for therapeutic purposes.

It may be pointed out, however, that tyrosine represents a normal protein constituent for which there is a definite nutritional and metabolic need. Reactions of the type described do not occur as long as the amounts of tyrosine introduced exceed by a wide margin the physiologic need of the organism for this amino acid.

# EFFECTS OF REPEATED INTRAVENOUS INJECTIONS OF LECITHIN IN RABBITS

## THE RELATIONSHIPS TO LIPOID STORAGE DISEASES AND TO HEMOLYTIC ANEMIAS

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Although the glycerophosphatides have not been specifically implicated in any lipoid storage disease, Kimmelstiel and Laas<sup>1</sup> and Epstein and Lorenz<sup>2</sup> found them to be increased in the tissues in Niemann-Pick disease and in Gaucher's disease, in addition to the lipoids primarily involved (the phosphatides classified as sphingomyelins and the galactolipid kerafin, respectively). Storage phenomena similar to those in Niemann-Pick disease were observed experimentally by von Beumer and Gruber<sup>3</sup> and Ferraro<sup>4</sup> following repeated intravenous injections of sphingomyelin, and much the same results, by Tompkins,<sup>5</sup> following similar injections of the ether-insoluble fraction of beef brain (a mixture of sphingomyelins and galactolipids).

Sjövall<sup>6</sup> expressed the belief that the glycerophosphatide lecithin, on the other hand, is utilized too rapidly in the normal organism to permit of storage except on administration of overwhelming amounts. He based this opinion in part on his failure to obtain storage phenomena following intravenous injections of this phosphatide in acute experiments, and he was supported in his opinion by similar failures on the part of von Beumer and Gruber<sup>3</sup> and Ferraro<sup>4</sup> in control studies which they performed with the glycerophosphatides. He did, however, obtain transitory accumulations of lipoid-containing cells following subcutaneous and intraperitoneal injections of lecithin, and Tompkins<sup>7</sup> found that subcutaneous injections of the glycerophosphatides (from brains and

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This material was presented before the American Society for Experimental Pathology.

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5. Tompkins, E. H.: *Am. J. Physiol.* **129**:481, 1940.

6. Sjövall, A.: *Acta path. et microbiol. Scandinav.* **12**:307, 1935.

7. Tompkins, E. H.: *Bull. Johns Hopkins Hosp.* **70**:55, 1942.

eggs), as well as of sphingomyelins, elicit local infiltrations of macrophages comparable to the specific cells of the lipid storage diseases. In view of these differences between the findings following intravenous and subcutaneous administration of lecithin, an investigation of the effects of intravenous injections repeated over long periods, in contrast to the more acute experiment of Sjövall,<sup>6</sup> seemed indicated.

# MATERIAL AND METHODS

Data concerning the purity of the lecithin injected, the dosages, the periods over which injections were given and the weights of the rabbits and of the organs

TABLE 1.—*Data on Duration of Experiments, Dosages, Weights and Hemopoietic Organs*

Rabbit AF Series	Form in Which Commercial Lecithin Was Given	Period Over Which Injections Were Given, Days	Dosages							Bone Marrow Activity	
			Total, Gm.	Maximum Daily		Weight, Gm.*			Estimated	Ratio to  Myeloid Elements Erythroid Elements	
				Gm.	Days Given	Rabbit	Spleen	Liver			
23	Untreated.....	5	2.88	1.00	1	4,120	1.8	152	++	1.14	
24	Untreated.....	74	37.94	1.00	6	4,400	8.6	112	++++	0.37	
26	Untreated.....	44	25.30	0.88	18	3,960	5.4	150	+++	0.79	
35	With the following Impurities extracted: water-soluble, acetone-soluble, ether-soluble	67	22.65	0.50	28	3,300	7.4	92	++++	0.66	
36		55	16.95	0.40	37	3,890	1.9	103	++++	0.91	
25	With cephalin ex- tracted in addition to substances re- ferred to above	57	25.43	0.79	14	4,560	5.2	200	+++	1.00	

\* The averages of the corresponding weights from 9 normal control rabbits killed in the same manner as the experimental animals are as follows:

Rabbit	3,800 Gm. (2,980 - 4,600)	Bone marrow
Spleen	2.4 Gm. (1.13 - 3.72)	Estimated activity ++
Liver	94.6 Gm. (68 - 110)	Ratio (M/E) 1.34 (0.8-2.4)

are presented in table 1. Commercial egg lecithin (Digestive Ferments Company) was employed merely because it is easy to obtain in bulk in relatively pure form. It was used without further purification for three experiments (rabbits 23, 24 and 26). For two others (rabbits 35 and 36) it was treated according to the method of MacLean<sup>8</sup> for removal of water-soluble impurities, followed by several extractions of the acetone-soluble and ether-insoluble lipoids (i. e., cholesterol, fatty acids, lysolecithin, sphingomyelins and galactolipids). For a sixth experiment (rabbit 25) it was further exhaustively extracted with alcohol for removal of cephalin.

The commercial product was kept in chloroform as a stock solution. The purified preparations were kept in ether as stock solutions, from which they were

8. MacLean, H.: J. Path. & Bact. **18**:490, 1914.

precipitated with acetone just before use in order to eliminate the possibility of inclusion of fatty acids split by storage (Boyd<sup>9</sup>). The stock solutions were stored in the ice chest. The materials for injection were measured fresh each time, freed from solvent by evaporation over a water bath, made into 5 per cent emulsions in 5 per cent dextrose and sterilized for two hours in a steam sterilizer. Adult nonpregnant female rabbits were used, and the injections were made in the aural vein. They were given at the rate of one every twenty-four hours, six days a week. The doses were increased gradually from 0.01 Gm. to constant levels varying from 0.4 to 1.0 Gm. daily (i. e., from 8 to 20 cc.). The injections were given slowly over a period of several minutes, and under those conditions, as Sjövall<sup>6</sup> found, no untoward effects were noted. The treatment by daily injections was continued over intervals of time which varied from five to seventy-four days. The animals were kept separately in large cages and were given a liberal diet of oats, carrots and a variety of greens, with water *ad libitum*. Normal controls were kept similarly. All were weighed once a week. All were clipped when the environmental temperature became sufficiently high to cause elevation of the white blood cell count in a control known to be susceptible. Pearce and Casey<sup>10</sup> demonstrated the seasonal effects which may occur in the blood cell counts of rabbits.

Total and differential white cell counts were made several times a week in all of the experiments. The differential counts were made on 200 cells by the supravital technic. The red cell counts were made infrequently at first. Since they revealed unexpected modifications, two experiments (rabbits 35 and 36) with lecithin from which contaminants known to influence hemolysis had been extracted were added to the series. In these two experiments detailed studies of the red cells were made at the time of each white cell count. Because of the great lability of rabbit's blood incident to circulatory fluctuations, all total counts were made in duplicate, with a third pipet in reserve for instances of disagreement. Trenner automatic pipets and Levy-Hausser counting chambers were used throughout. Except when the blood was followed over a twenty-four hour period after a single injection, the sample for study was always drawn a few hours before an injection.

In the two experiments with detailed study of the red cells the following procedures were carried out: total red cell counts (Hayem's solution), platelet counts (direct counts from the same counting chamber specimens used for the red cell counts), reticulocyte counts (wet counts on 1,000 red cells from pipet contents diluted with 1 per cent brilliant cresyl blue in saline solution and shaken automatically), hemoglobin determinations (Klett hemoglobinometer, calibrated against a standard which establishes 100 per cent as equivalent to 17 Gm. of hemoglobin in 100 cc. of blood), duplicate hematocrit determinations (Van Allen hematocrit tubes centrifuged for forty-five minutes at 2,700 revolutions per minute) and measurements of cell diameters. The diameters were measured on 50 cells in supravital smears as they passed across the field of an ocular micrometer. While this represents an unsatisfactorily small number of cells per count, it is believed that the frequency of the counts, together with the grouping of them into larger weekly units (table 2), results in information as valuable as that which could be obtained from counts of larger numbers of cells made at less frequent intervals.

The fragility of the red cells to water was measured from time to time in these two experiments by removing drops of blood from an ear by fine glass rods

9. Boyd, E. M.: *J. Biol. Chem.* **121**:485, 1937.

10. Pearce, L., and Casey, A. E.: *J. Exper. Med.* **52**:39, 1930.

and immediately submerging them in small tubes containing standard dilutions of saline solution. This method was chosen in order to avoid the stimulus to erythropoiesis which might result from frequent removal of amounts of blood large enough for fragility tests made in the usual manner. The control tests agreed within normal limits, not only at the time of a single determination but from day to day.

The icteric index was determined in these two experiments on 1 to 2 cc. of blood withdrawn from an aural vein before an injection. The determinations were

TABLE 2.—*Weekly Averages of the Data Concerning the Erythrocytes of the Two Rabbits (35 and 36) That Were Given Daily Injections of Lecithin from Which Contaminating Hemolysins Had Been Extracted*

Interval of Time	Average Daily Dose of Lecithin, Gm.	Total Count		Hemoglobin Content, Percentage	Hematocrit Reading, Percentage	Average Cell Volume, Cubic Microns	Average Cell Diameter, Microns	Indexes *		Average Cell Thickness, Microns †
		Red Blood Cells	Reticulo-eytes					Color	Vol-ume	
Rabbit 35										
May 5-16	Control	5,778,333	108,460	59	40.7	70.5	8.8	1.0	1.00	1.19
to 24	0.16	5,423,333	83,283	60	41.6	76.7	8.0	1.1	1.07	1.55
to 30	0.27	5,247,500	139,830	57	35.5	67.6	8.2	1.1	0.95	1.32
to June 5	0.30	5,586,500	147,869	57	37.5	67.2	8.9	1.0	0.94	1.10
to 13	0.39	4,530,000	217,796	55	30.3	66.9	8.4	1.2	0.94	1.25
to 19	0.46	4,462,500	524,805	51	28.8	64.5	9.0	1.1	0.90	1.05
to 27	0.50	4,083,333	437,082	47	25.7	62.0	8.5	1.1	0.88	1.11
to July 4	0.50	4,362,500	390,575	47	28.2	64.6	8.5	1.1	0.91	1.18
to 11	0.50	4,147,500	257,223	48	27.8	66.9	...	1.1	0.94	....
to 17	0.50	4,232,500	449,393	45	26.5	62.4	9.1	1.0	0.88	1.00
to 21	0.50	4,737,500	365,873	50	28.5	60.2	8.0	1.0	0.84	1.25
Rabbit 36										
May 3-16	Control	5,478,333	59,563	59	33.6	61.3	8.0	1.0	1.00	1.22
to 20	0.15	5,290,000	52,900	58	33.1	62.6	7.3	1.0	1.00	1.50
to 26	0.29	5,062,500	105,777	56	....	....	7.3	1.0	....	....
to 31	0.39	4,405,000	50,410	55	28.4	64.5	7.0	1.2	1.03	1.70
to June 6	0.40	4,610,000	191,270	51	26.5	57.5	7.8	1.0	0.92	1.20
to 14	0.40	4,256,666	224,058	49	25.8	60.7	8.5	1.1	0.97	1.10
to 20	0.40	4,275,000	283,375	50	27.0	63.2	8.5	1.1	1.01	1.15
to 28	0.40	4,071,666	358,260	47	25.1	61.6	8.2	1.1	0.99	1.20
to July 3	0.40	4,230,000	155,520	44	26.8	63.3	8.5	1.0	1.01	1.12
to 10	0.40	4,476,666	196,258	47	27.5	61.4	8.4	1.0	0.98	1.13

\* The indexes are based on the control values for each rabbit as normal for that rabbit.

† The thickness was estimated from the chart devised by von Boras for the calculation of the thickness of human erythrocytes, as reproduced by R. L. Haden (*Principles of Hematology*, Lea & Febiger, Philadelphia, 1940).

made only at infrequent intervals in order to avoid the effects of too frequent withdrawal of blood. The serum was treated with two volumes of absolute alcohol, centrifuged and the supernatant fluid matched against dichromate standards. The urine (either the first voiding after an injection, or the twenty-four hour voidings) was also frequently tested in these two experiments for specific gravity, sugar (Benedict's qualitative solution), albumin (nitric acid and heat), blood (guaiac test) and bile pigments (Gmelin's and Rosenbach's methods).

All of the animals were killed eighteen to twenty-four hours after the last injection by intravenous administration of soluble pentobarbital. The inferior vena cava was cut at once and the blood allowed to drain with the animal held upright.

The spleen and the liver were then weighed (table 1), and all organs were examined for gross lesions. The tissues were saved routinely in Bouin's fixative. Blocks of the erythropoietic organs were also saved in Kingsley's<sup>11</sup> and Müller's fixatives, and scrapings were studied supravitaly. Staining for fat was usually done on sections from the blocks fixed in Bouin's solution. All sections were stained routinely with hematoxylin and eosin; Kingsley's stain<sup>12</sup> was employed, in addition for the erythropoietic tissues, including the sections of marrow used for counts.<sup>13</sup> Histochemical tests for iron (Key's technic for the prussian blue reaction) were carried out both before and after unmasking. Unmasking was accomplished both with 3 per cent nitric acid in alcohol and with trypsin employed at different intervals of time according to the section.

#### EXPERIMENTAL DATA

Daily doses of as much as 0.8 Gm. of either the commercial or the purified lecithin were well tolerated over long periods. The animals retained their weight or gained and seemed active and normal in every respect. Greater doses were given only in the case of the commercial lecithin without further purification. One animal (24) that had tolerated smaller doses for weeks became asthenic and lost appetite after three or four doses of 1.0 Gm. One (23) that was not known to be pregnant was found moribund eighteen hours after a single injection of 1.0 Gm., and autopsy revealed dead fetuses. Both of these animals had marked central fatty necrosis of the liver, in contrast to the absence of demonstrable fat in the livers of the other animals of the series.

*White Blood Cells.*—It is planned to present the data obtained from serial counts following single injections in these experiments in greater detail elsewhere in order that they may serve as control counts for a different series of studies. It is sufficient for the purposes of this report to state that immediately after an injection, whether in an animal previously given injections or not, a transient and slight increase of the total white cell count occurred, involving both neutrophils and lymphocytes, but accompanied by definite monocytopenia. Within about one and a half hours after the injection, the lymphocytes also decreased slightly. Usually the counts gradually returned to the preinjection levels within twelve hours.

While the immediate effects of the injections were circulatory in nature and transient, the daily curves usually showed gradual changes, irrespective of the purity of the material injected. These consisted of moderate increases in the total white cell counts, dependent largely on slight increases in lymphocytes and increases of variable degrees in monocytes. The elevation of monocytes became evident between the third and fourteenth days of the injections, i. e., with daily doses of lecithin which varied from 0.2 to 0.5 Gm. The elevation of lymphocytes was slightly more sluggish in making an appearance. These changes in the white cell counts are quite in agreement with those found previously in a series of control counts made on rabbits treated with lecithin before inoculation with tubercle bacilli.<sup>14</sup> When these studies are coupled with those studies, it seems definite that intravenous injections of egg lecithin stimulate the circulating mononuclears, usually affecting both lymphocytes and monocytes, but the latter more consistently than

11. Kingsley, D. M.: *Folia haemat.* **57**:87, 1937.

12. Kingsley, D. M.: *Stain Technol.* **10**:127, 1935.

13. Epstein, R. D., and Tompkins, E. H.: A Comparison of Techniques for the Differential Counting of Bone Marrow (of the Guinea Pig), *Am. J. Med. Sc.*, to be published.

14. Tompkins, E. H.: *Am. Rev. Tuberc.* **33**:625, 1936.

the former. Figures 1 and 2 present the blood curves of the 2 rabbits (35 and 36) that received daily injections of lecithin extracted for water-soluble, acetone-soluble and ether-insoluble impurities. Platelet counts were also made for these 2 animals but are not charted. They increased slightly by the end of the second week and remained elevated thereafter.

*Red Blood Cells.*—By the third week of the injections the red cell counts were found to have decreased about 1,000,000 cells in each experiment, and there was a corresponding decrease in hemoglobin, with nucleated red cells appearing intermittently. The daily dose of lecithin by that time varied from 0.2 to 0.6 Gm. The counts and hemoglobin levels remained fairly stationary thereafter.

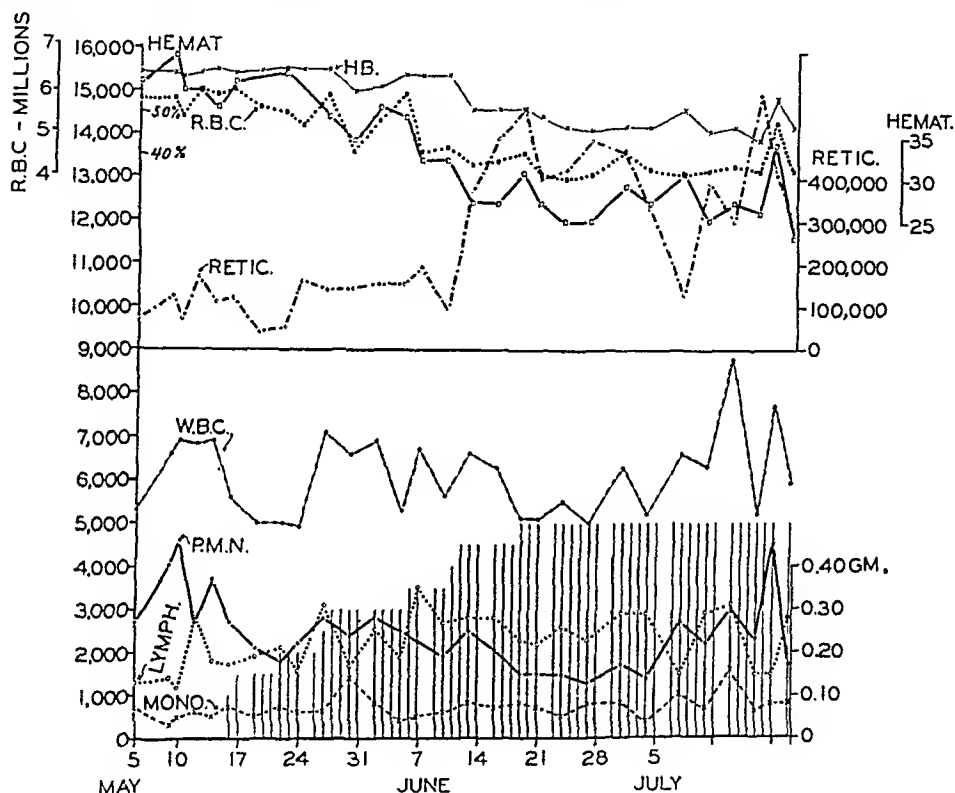


Fig. 1 (rabbit 35).—Curves of the total, reticulocyte and differential (absolute numbers) blood counts and of the hemoglobin and hematocrit readings. The different curves are correlated with the daily injections of lecithin.

In this figure and in figure 2 the white blood cell curves and the daily doses of lecithin are shown below the horizontal line (---, white cell counts; —, neutrophils; o....o lymphocytes; x---x monocytes). The scale at the left represents thousands per cubic millimeter.

Above the horizontal line, the red cell curves are shown (■....■ red cell counts, with scale at the upper far left—millions per cubic millimeter; o....o reticulocyte counts, with scale at the right—hundred thousand per cubic millimeter; x — x hemoglobin readings, with scale at the upper inner left—grams per hundred cubic centimeters; □ — □ hematocrit readings, with scale at the far right—volumes per cent).

It was because of these unexpected findings that the two experiments (rabbits 35 and 36) were carried out with lecithin after extraction of fatty acids, lysolecithin and cholesterol, and with more detailed studies of the red cells. Table 2

and figures 1 and 2 present most of the data of these two experiments. On the fourteenth and thirteenth days of the injections, respectively, i. e., with daily doses of 0.3 and 0.4 Gm. of lecithin, the red cell counts and the hematocrit and hemoglobin readings began to decrease slowly. Reticulocytosis developed by the end of the third week of the injections (when the daily doses were 0.45 and 0.40 Gm., respectively) and increased precipitantly to remain at elevated levels throughout the remainder of the experiment. The red cell counts and the hematocrit and hemoglobin values then became stationary at the levels they had attained before this occurred. Nucleated red cells appeared in the circulation in small numbers. The erythrocytes themselves appeared unusually flat and malleable in the supravital smears, with a

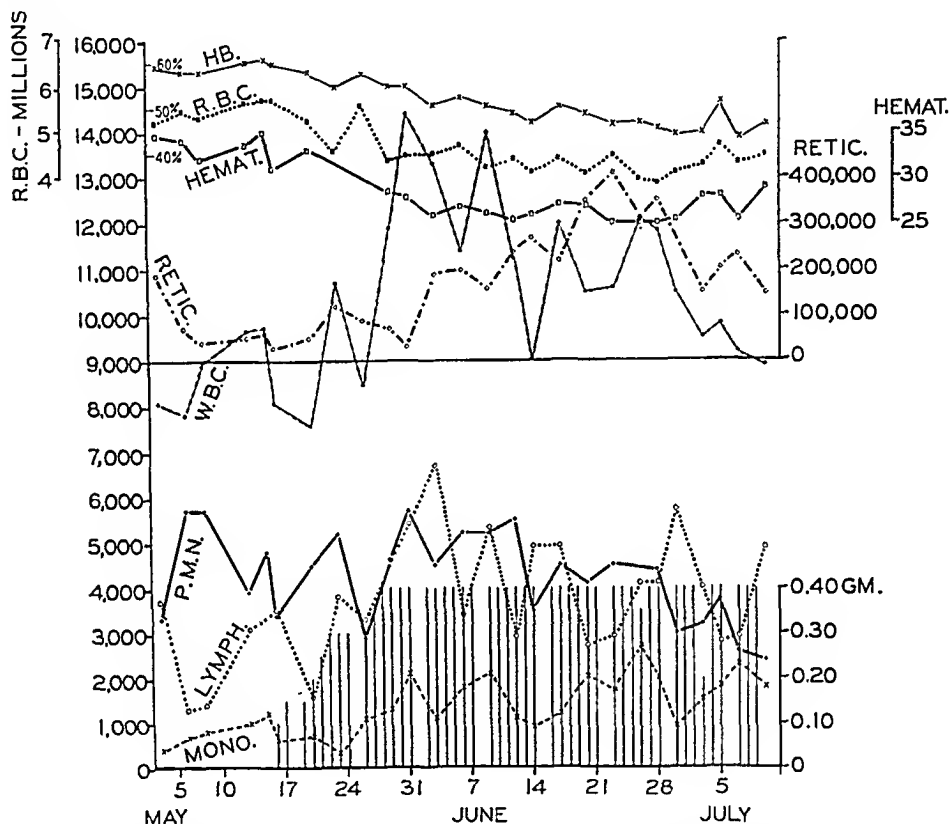


Fig. 2 (rabbit 36).—Curves of the total, reticulocyte and differential (absolute numbers) blood counts and of the hemoglobin and hematocrit readings. The different curves are correlated with the daily injections of lecithin.

tendency toward leaflike and sickle-shaped forms (figs. 3 *A*, *B* and *C*). Such forms often appeared in clusters in the supravital smears. They did not increase in number, however, when the smears were left in the hot box overnight. Variability in size became evident after the onset of reticulocytosis. The distribution curves of the diameters of erythrocytes (Price-Jones) were at first shifted slightly toward the left only but later toward both the left and the right. The average diameters, based on all of the measurements for each week of the experiment, also reflect these changes. The volume indexes increased at first and later decreased. The color indexes remained at unity or above. The weekly averages of these various data are recorded in table 2.

Although the fragility readings did not deviate beyond normal limits, they were consistently higher than those obtained before the injections were started, and the



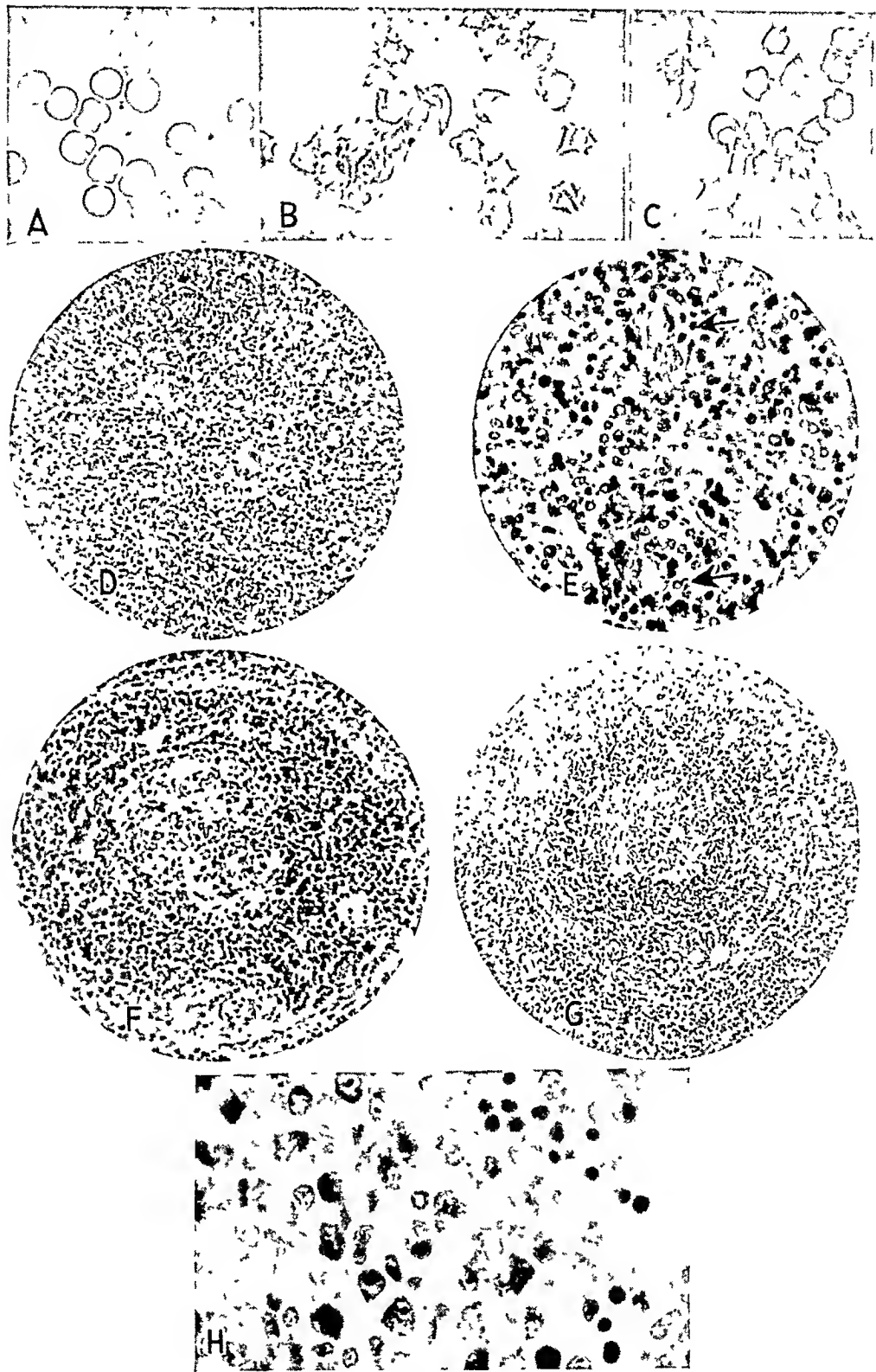


Figure 3  
(See legend on opposite page)

differences between the points of beginning and complete hemolysis were slightly greater. The icteric indexes increased slightly, 6 units being the maximum reading at any time. The urine was consistently negative to the guaiac test, was rarely faintly positive to the Rosenbach test for bile pigment and contained traces of sugar and albumin only during the last two weeks of the experiment, when the environmental temperatures were exceptionally elevated.

*Pathologic Changes.*—The general nutrition was excellent, with abundant subcutaneous and intra-abdominal fat. The fur was thick and silky, and the corneas were normal in appearance.

The major findings were in the hemopoietic organs and were relatively proportional to the maximum daily dose of lecithin (table 1). Daily amounts greater than 0.4 Gm. were apparently necessary for gross changes, although hematologic and morphologic changes occurred with that dose. The femoral marrow was dark red and voluminous but not appreciably firmer than normal. The lymph nodes appeared normal grossly. The spleen was dark purple, firm, unevered when cut and noticeably smooth in texture with inconspicuous markings, and weighed about three times the average for the normal spleen under the same conditions. The liver weighed more than normal and was somewhat darker than normal in color, except in the rabbits which received the maximum doses of crude commercial lecithin (1.0 Gm. daily). In those it was pale and fatty.

The sections of the hemopoietic organs presented alterations of considerable interest, especially those of the spleen. The latter will therefore be described in detail and the others only as related to them.

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#### EXPLANATION OF FIGURE 3

*A* (rabbit 35), supravital blood smear (two hours old) on the fifty-seventh day of the experimental injections. The erythrocytes appeared abnormally mal-leable with a marked tendency toward leaflike and sickle-shaped forms. Two leaflike forms are illustrated.  $\times 718$ .

*B* and *C* (rabbit 36), supravital blood smears (two hours old) on the fifty-seventh day of the experimental injections. Elongated and sickle-shaped erythrocytes of the type illustrated frequently occurred in clusters.  $\times 718$ .

*D* (rabbit 35), spleen. The splenic pulp was crowded with macrophages and exhibited extramedullary hemopoiesis. The lymphoid nodules were markedly reduced in size and numbers, and the sinusoids were greatly compressed.  $\times 112$ .

*E*, higher magnification of *D*. The marked infiltration of macrophages and the depletion of lymphoid tissue are evident. The arrows point to arteries about which are the remnants of lymphoid nodules.  $\times 322$ .

*F* (rabbit 35), spleen (different area from that shown in *D* and *E*). A malpighian corpuscle is seen. A large mass of homogeneous acidophilic material is shown within the concentric sinusoids of the corpuscle (lower part of the figure). Smaller masses are scattered elsewhere around the periphery of the nodule.  $\times 170$ .

*G* (rabbit 25), spleen. A malpighian corpuscle is shown. Characteristic masses of homogeneous acidophilic material are shown within the concentric sinusoids of the corpuscle.  $\times 73$ .

*H* (rabbit 35), spleen. Same as *D* and *E*. Extramedullary hemopoiesis occurred diffusely. A cord densely infiltrated with macrophages and with myeloid cells is shown bordering a sinusoid in which there is active erythropoiesis.  $\times 718$ .

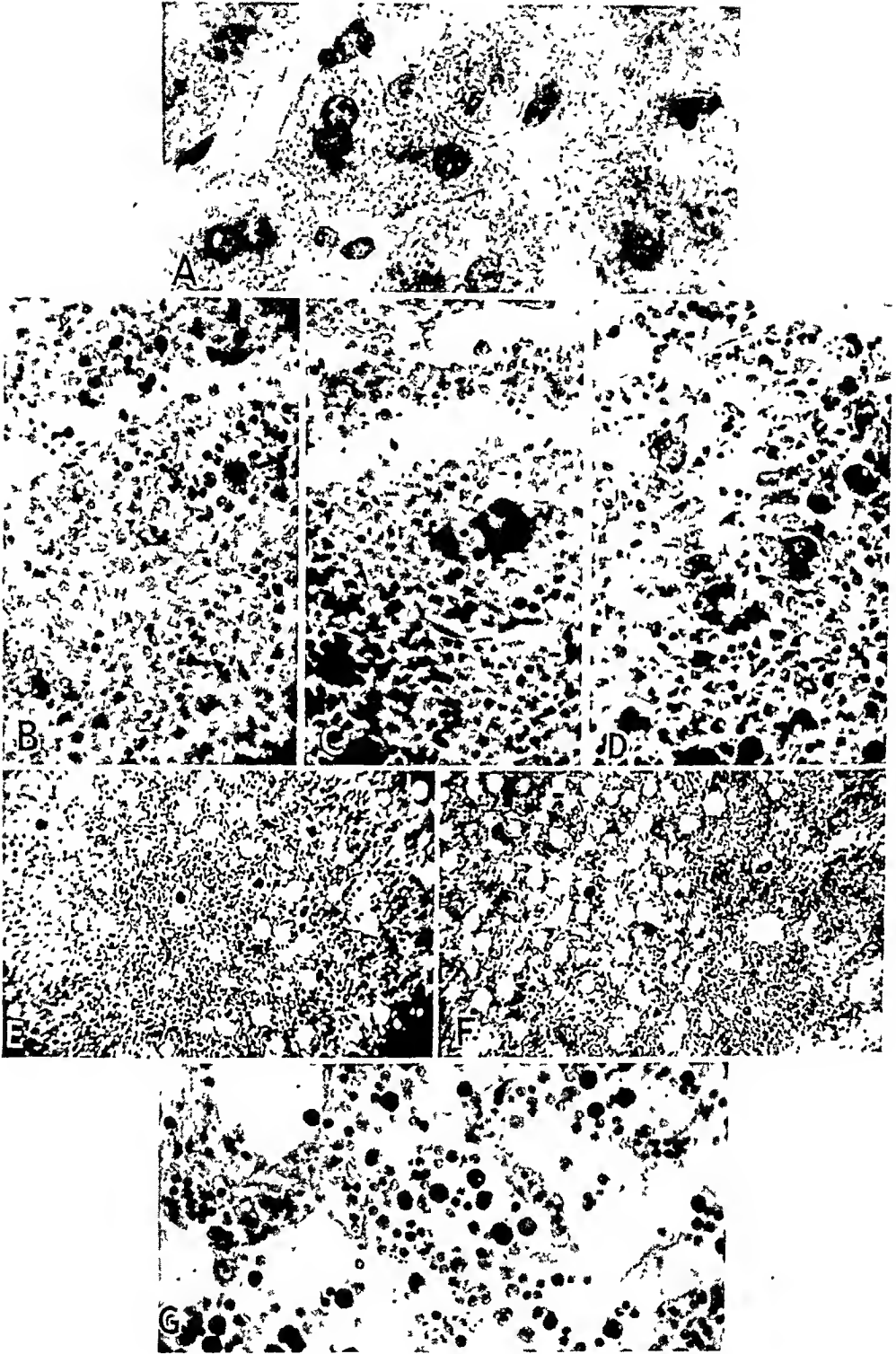


Figure 4

(See legend on opposite page)

The splenic tissue was densely packed with cells, with a consequent inconspicuousness of the sinusoids, both because they themselves shared in the infiltrations and because they were compressed by the markedly infiltrated and thickened cords between them (fig. 3 *D*). The compactness of the infiltrations, coupled with a reduction of the malpighian corpuscles to the point of obliteration in some cases, lent a homogeneity to the picture that was often difficult to recognize as splenic (fig. 3 *E*).

Where the corpuscles were not yet reduced practically to arterioles and scattered lymphocytes, they were frequently surrounded by broad bands of homogeneous acid-staining material (figs. 3 *F* and *G*). This material was situated within the concentric peripheral sinusoids and was similar to that which Rich<sup>15</sup> noted in the spleen in cases of sickle cell anemia.

The cells which packed the cords and sinusoids so densely and which replaced the areas originally occupied by lymphoid tissue consisted of a mixture, in which, however, cells of one type invariably occurred in great abundance. These cells,

15. Rich, A. R.: Bull. Johns Hopkins Hosp. 43:398, 1928.

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#### EXPLANATION OF FIGURE 4

*A* (rabbit 25), liver stained with sudan IV and sudan III. The parenchymal cells contained little fat. The Kupffer cells were increased in number and varied in contents; many were swollen with large deposits which stained an apricot color (two such are illustrated); none were found with the deep color characteristic of neutral fats.  $\times 718$ .

*B* (rabbit 35), spleen stained by Key's technic for the prussian blue reaction to iron. The sinusoids (upper part of the illustration) were filled with material which stained dark blue. The macrophages of the pulp, on the other hand, rarely reacted to the histochemical test. At times the deposits in them stained faintly blue, but the greater proportion retained their inherent brownish yellow color. Darkly stained deposits were extremely rare.  $\times 322$ .

*C*, same as *B*. The section was treated with 3 per cent nitric acid in alcohol before it was stained for iron. This treatment unmasked large amounts of iron not stainable previously. The contents of the sinusoids stained much the same as in *B*, but the deposits in the macrophages stained blue far more frequently. Many were still brownish yellow, but the majority were tinted with variable degrees of blue, so that they ranged in color from pale green, through the pale and deeper blues, to a few very dark blue ones.  $\times 322$ .

*D*, same as *B*. The section was treated with trypsin before being stained for iron. The amount of iron unmasked by this treatment was still greater than that shown in *C*. The sinusoids appeared as in *B* and *C*. The macrophages contained few deposits that were still brownish yellow. Most of the deposits varied in color from medium to dark blue.  $\times 322$ .

*E* (rabbit 35), center of the bone marrow from the femur, showing marked hyperplasia. Kingsley's technic;  $\times 73$ .

*F* (rabbit 36), center of bone marrow from the femur, showing hyperplasia of a slightly less degree than that illustrated in *E*. Kingsley's technic;  $\times 73$ .

*G* (rabbit 35), mesenteric node. The sinusoids were loaded in increasing numbers from cortex to medulla with large basophilic nongranular cells. The cytoplasm of most of these cells was intensely blue, but paler cells were intermingled throughout. Kingsley's technic;  $\times 322$ .

which were always present regardless of the proportions of the other types and which will be shown to have been present also in all of the hemopoietic tissues, were macrophages. They varied considerably in size. Their cytoplasm always contained an abundance of flocculent deposits, which had an intrinsic brownish yellow hue, and also often contained cellular and nuclear fragments but rarely whole erythrocytes. Binucleated and giant cells were never met. The brownish, flocculent contents of the macrophages proved difficult of analysis. In agreement with the findings of Sjövall<sup>6</sup> in rabbits which had been given lecithin intravenously within twenty-four hours before being put to death they stained an apricot hue with sudan IV and sudan III, and bright red staining was rare. They stained an intense dark blue or dark green with nile blue sulfate but did not stain with osmic acid vapor. Their capacity to stain with nile blue sulfate was unaffected by treatment with various fat solvents (xylene, alcohol, combinations of alcohol, chloroform and ether), and they remained apparently unchanged. This material took acid dyes with difficulty. It stained faintly for iron (fig. 4 *B*) and was therefore presumably hemoglobiniferous in origin, though not in the form of whole erythrocytes and obviously not in the deeply stainable form usually met in splenocytes. In fact, deep staining occurred only in the sinusoids, where innumerable strings and globules, apparently free, were stained dark blue. Prolonged treatment of the sections with nitric acid before staining for iron resulted in considerably deeper staining of the deposits in the macrophages with a variety of hues that ranged in intensity from indigo to pale blue or green (fig. 4 *C*). There remained, however, a variable number of brownish yellow deposits still unmasked. Many of these could be more successfully unmasked by treatment of the sections with trypsin for variable periods before staining for iron (fig. 4 *D*). It is obvious, therefore, that the phagocytosis of whole erythrocytes and the presence of unmasked iron in the spleen were rare except in the sinusoids, but that the macrophages were nevertheless filled with quantities of flocculent material in which masked iron could be demonstrated and which therefore most probably represented fresh fragments of erythrocytes.

In addition to the macrophages which jammed the cords and sinusoids of the spleen, a variety of hemopoietic entities also usually occurred (fig. 3 *H*). These were relatively infrequent in the animal (36) with the smallest daily dose and increased almost proportionately to the dose. Nucleated red cells occurred in great numbers within both sinusoids and cords. They tended to occur in islands and at various stages of development. Myelocytes were frequent in the cords and rare in the sinusoids. They also tended to occur in clusters. Megakaryocytes were also frequent in the cords of the spleen with the greatest degree of hemopoietic activity. Finally, cells of a large type, far more abundant in the nodes (fig. 4 *G*) than in the spleen, were met infrequently in the experiments with the smaller injections but relatively often in those with the larger ones. These large cells were strikingly clear and glassy in supravital preparations and did not stain with neutral red. At times they contained very coarse mitochondria. With Kingsley's stain, their cytoplasm varied from intense basophilia to a clear pale blue, and the nuclei varied correspondingly from stippled to vesicular. Normal reticular cells, as well as the lymphocytes, became relatively decreased as these different forms increased within the splenic tissue. No evidence was obtained of necrosis or fibrosis.

Apart from the livers of the animals given the largest doses of crude lecithin, in which central fatty necrosis occurred, fat was rarely demonstrated in the parenchymal cells. The Kupffer cells (fig. 4 *A*) were often hypertrophied, some-

what increased in number and stained like the macrophages described in the spleen except that they contained relatively less material that stained for iron. Free macrophages and hemopoietic elements as described in the spleen were met infrequently in the hepatic sinusoids.

The nodes, particularly the mesenteric ones, contained many clusters of macrophages similar in all respects to those in the spleen. In addition, the reticular cells often contained small droplets similar to the contents of the macrophages. Erythropoiesis and myelopoiesis were never observed in the nodes. On the other hand, basophilic cells similar to those which were found in moderate numbers in the spleens occurred in overwhelming numbers in the nodes (fig. 4 G). They lay in the sinusoids in increasing numbers from cortex to hilus.

The marrow was hyperplastic with considerable reduction of fat spaces as compared with the normal (fig. 4 E and F). Usually the hyperplasia extended throughout the entire section. Counts made on sections stained by Kingsley's technic revealed a relative increase in erythroid over myeloid elements as indicated by low myeloid-erythroid ratios as compared with the normal (table 1). Macrophages similar to those described in the spleen occurred somewhat in proportion to the size of the daily dose of lecithin.

The lungs appeared normal grossly, but macrophages occurred in greater abundance than normal in the septums and also, rarely, as diffuse accumulations, with constriction of neighboring alveolar spaces. Unlike the macrophages in other sites, these cells when stained supravitaly contained the rather small vacuoles which are characteristic of pulmonary phagocytes normally, and they rarely contained brownish yellow deposits. Periarteriolar accumulations of eosinophils also occurred in varying intensities, irrespective of the purity of the lecithin administered.

The heart appeared large and flabby, with a rather firm left ventricle and a large right one.

The kidneys of the animals which received the crude commercial product contained fine green petechiae or scarring of the cortices. The kidneys of the other rabbits were normal in appearance. The characteristic macrophages occurred sporadically along the tubules or in the glomeruli.

The adrenals appeared smaller than normal, and the sections revealed abnormalities of the zona fasciculata and the zona reticularis which varied considerably in degree. In the extreme degree of change (rabbit 35) the zona fasciculata was abnormally constricted and lacking in fat, while the zona reticularis was abnormally broad because of the hypertrophy and unusually abundant lipoid content of its cells. In this form the changes resembled the findings which Takéda<sup>16</sup> obtained in a proportion of his experiments with guinea pigs. In less involved glands the cells of the zona fasciculata appeared normal and seemed to contain the normal amount of fat, but nests of large cells, with small eccentric nuclei and unusually irregular deposits of neutral fat, occurred in the inner zone and impinged on the zona reticularis more abundantly than in the adrenals from the controls. Macrophages occurred sporadically.

No consistent changes were found in the ovaries, the uterus or the mammary tissues other than the frequent appearance of clusters of the characteristic macrophages in the adipose tissue surrounding the mammary glands.

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## COMMENT

The findings are obviously different from those of the recognized lipid storage diseases with their characteristic infiltrations of huge cells filled with material extractable by lipid solvents and therefore vacuolated and foamy in appearance in fixed tissues. These cells were filled with a substance which reacted to fat stains but which was not extracted in large enough amounts by fat solvents to leave appreciable space in fixed tissues, and which seemed to be linked with hemoglobin-bearing material in such a manner that the iron could be demonstrated only by unmasking with nitric acid or trypsin. These cells were smaller than those in the known lipid storage diseases. The cells obtained by subcutaneous injections of lecithin were also smaller than those obtained by similar injections of the lipids concerned in the known storage diseases.<sup>7</sup>

Yet in other respects the experimental pathologic condition bears close resemblances to the lipid storage diseases. A lipid was administered in large amounts. The same organs were involved as in the lipid storage diseases and, to a large extent, in the same manner. The marrow, the lymph nodes, the spleen and the liver contained far greater numbers of macrophages than normal. These phagocytes filled the same spaces in the spleen as in the lipid storage diseases, i. e., the cords, and there was the same tendency toward depletion of lymphoid tissue. The spleen was greatly enlarged, and there was extramedullary hemopoiesis. Sjövall<sup>6</sup> also observed the invasion of the sinusoids and cords of the spleen by phagocytic cells in the two experiments in which he gave repeated injections of lecithin. He considered these cells to be "lipophages" and commented on the parallelism with the storage phenomena of Niemann-Pick disease. In their content of iron these cells also resemble the features often observed in cases of Gaucher's disease.

From another point of view, however, the findings may be regarded as typical of those of any chronic hemolytic anemia. There was gradual decrease in the number of erythrocytes, the hemoglobin content and the hematocrit reading. These changes were ultimately checked by increased erythropoiesis, as indicated by continued reticulocytosis, the occurrence of nucleated red cells in circulation and the extramedullary activity of the spleen in addition to accelerated erythropoiesis in the marrow. These changes were accompanied by spreading of the distribution curves of diameters of erythrocytes (Price-Jones) to both right and left, by slight decreases in volume index without change in color index, and by slight increases in icteric index. Macrophages filled with hemoglobin-bearing particles occurred in great numbers in all of the hemopoietic tissues.

The appearance of the splenic nodules, the malleability and normal fragility of the erythrocytes and the marked erythrophagocytosis, or at least the phagocytosis of fragments of erythrocytes, are features which bear close resemblances to the findings in early cases of that particular form of hemolytic anemia met in sickle cell anemia (Huck<sup>17</sup>; Sydenstricker<sup>18</sup>; Diggs and Ching<sup>19</sup>; Graham and McCarty<sup>20</sup>). The amount of demonstrable iron in the spleen in this disease may be small as compared with the degree of erythrophagocytosis.<sup>21</sup> Peabody and Broun<sup>22</sup> made the same observation in cases of pernicious anemia, and it was also made in these experiments until methods of unmasking were employed.

It seems, therefore, that one is dealing with reactions in the tissues that may be regarded as an admixture of those of a lipid storage disease and those of hemolytic anemia, both elicited entirely by the administration of a glycerophosphatide.

If one attempts to analyze the basis of the hemolytic features of the picture several possibilities present themselves. Lecithin stimulates the production of macrophages when injected subcutaneously.<sup>7</sup> It seems from these experiments that it does this also when administered intravenously. The mooted question arises as to whether abnormal destruction of erythrocytes may occur due entirely to overactivity of the reticulo-endothelial system. That this is not the chief explanation in this instance seems likely in view of the fact that phagocytosis of whole erythrocytes was obviously rare and that, likewise, it was rarely observed in the earlier experiments with subcutaneous injections of lecithin.<sup>7</sup> Furthermore, the tissues from those experiments have been restudied with the technics for the demonstration of iron that were employed in the present series, and they have yielded positive results so rarely as to lead to the conclusion that such instances represent merely the response to hemorrhage occurring occasionally at the time of the subcutaneous injections.

That direct hemolysis with liberation of free hemoglobin into the blood stream may have occurred incident to the injections and given rise to the hemolytic features of the picture also seems unlikely in view of the lack of positive evidence to that effect. Except in the experiments with crude commercial lecithin from which fatty acids and other possible contaminating hemolysins had not been extracted, there were no observable reactions on the part of the animals, no positive results from tests for blood in the urine and no findings in the tissues suggestive of direct hemolysis and liberation of free hemoglobin.

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17. Huck, J. G.: *Bull. Johns Hopkins Hosp.* **34**:335, 1923.

18. Sydenstricker, V. P.: *South. M. J.* **17**:177, 1924.

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20. Graham, G. S., and McCarty, S. H.: *South. M. J.* **23**:598, 1930.

21. Diggs and Ching.<sup>19</sup> Graham and McCarty.<sup>20</sup>

22. Peabody, F. W., and Broun, G. O.: *Am. J. Path.* **1**:169, 1925.



That the lecithin, on the other hand, affected the erythrocytes in such manner that they were more subject to fragmentation and phagocytosis than normal seems likely. The character of the inclusions within the macrophages supports this concept; these inclusions obviously were not whole erythrocytes; yet they had characteristics of those elements. The slight tendency toward sphericity which was found in the circulating erythrocytes supports the same concept. The measurements of diameter were made routinely many hours after the injections, and it is possible that even greater distortion of the cells occurred earlier. Experimental evidence supports the probability that phospholipids in circulation are adsorbed on erythrocytes (Bloor <sup>23</sup>); Ponder <sup>24</sup> showed that erythrocytes become spheroidal in suspensions of lecithin; various investigators (Haden <sup>25</sup>; Dameshek <sup>26</sup>; Castle and Daland <sup>27</sup>; Singer <sup>28</sup>) have demonstrated that sphericity is associated with increased fragility, and it seems probable that the earlier studies concerning the antagonistic effects of lecithin and cholesterol on the "permeability" of erythrocytes to various hemolytic agents are explainable on a similar basis (Brinkman and von Dam <sup>29</sup>; Kimmelstiel <sup>30</sup>; Spranger <sup>31</sup>). While the fragility of the erythrocytes in these experiments did not exceed normal limits, the fact that the resistance was consistently slightly less after the injections started than in the control measurements is probably significant of some degree of increased fragility. This is especially true in view of the great number of relatively resistant reticulocytes in circulation. Dameshek and Schwartz <sup>32</sup> called attention to the fact that measurements of fragility, as well as those of average cell diameter, represent a composite of the measurements of the more resistant young cells and of the less resistant spheroidal ones.

The findings, therefore, support the concept that the erythrocytes were rendered abnormally subject to fragmentation by the injected lecithin. Fragmentation was undoubtedly further aided by the conditions within the spleen. The cords were greatly hypertrophied by the infiltration of macrophages and the hemopoiesis within them. The sinusoids were correspondingly compressed and, furthermore, were con-

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gested with exceptional numbers of cells similar to those in the cords. The erythrocytes must have met considerable impediment within them, and this, combined with the fact that they were already fragile by virtue of the action of the lecithin on them, may be supposed to have subjected them to an unusual degree of trauma.

How long the direct effects of lecithin on erythrocytes may have lasted between injections is entirely subject to surmise as far as these experiments are concerned. Sjövall<sup>6</sup> expressed the opinion that lecithin is so rapidly absorbed that its effects are inevitably transitory. The chemical studies of Pasternak and Page<sup>33</sup> with injections of cephalin and those of Haven and Bale<sup>34</sup> with labeled phosphatide would at first thought suggest the same probability. These observers found that the glycerophosphatides which they used were removed from the circulation within one half to two hours unless given in far greater amounts than in these studies. However, they found these materials to be deposited largely in the spleen and the liver at first, with a gradual shift later from those sites into other areas. These observations therefore lead to the suggestion that the local effects of lecithin on erythrocytes in the present experiments probably continued far longer in those organs than in the general circulation, with the result that the effects in those sites were practically chronic.

From this discussion, therefore, the conclusions seem justified (1) that repeated intravenous injections of lecithin result in tissue changes which are representative at one and the same time of those in lipid storage diseases and those due to destruction of erythrocytes, (2) that the former are characterized by stimulation of the reticuloendothelial system but are modified by changes which are due to the destruction of erythrocytes, and (3) that the destruction of erythrocytes is due to fragmentation which is facilitated by the direct effect of lecithin on the resistance of erythrocytes together with unusual stress exerted on them in the splenic and other sinusoidal areas.

#### SUMMARY

Repeated intravenous injections of lecithin in sufficient amounts cause:

1. A generalized infiltration of macrophages, which contain both lipoids and fragments of erythrocytes but which stain for iron only after unmasking.
2. An increase in the white blood cell count, dependent largely on increases in the numbers of lymphocytes and monocytes.

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34. Haven, F. L., and Bale, W. F.: *J. Biol. Chem.* **129**:23, 1939.

3. A decrease in the red blood cell count, accompanied by a decrease of the hemoglobin and hematocrit readings, a slight decrease in volume index, a tendency toward decreased resistance, abnormal malleability of the erythrocytes, a slight increase in the icteric index and a normal color index. These changes eventually are checked and held stationary by continued reticulocytosis, accompanied by circulation of nucleated red cells and spacing of the distribution curves of diameters of erythrocytes (Price-Jones) to the right and the left.
4. Hyperplasia of the bone marrow with increased erythropoiesis, decrease of the myeloid-erythroid ratios and infiltrations of the characteristic macrophages.
5. Splenomegaly associated with (*a*) massive infiltrations of the characteristic macrophages, (*b*) hemopoiesis, (*c*) deposition of acidophilic bands about the peripheries of the malpighian corpuscles and depletion of the corpuscles and (*d*) constriction and congestion of the sinusoids.
6. Infiltrations of young basophilic cells in the lymphoid tissues.

The findings are discussed from the standpoint of their mutual relationships both to the lipoid storage diseases and to hemolytic anemias.

The influence of lecithin on the resistance of erythrocytes and the exposure of the erythrocytes to an exceptional degree of trauma in the spleen are offered in explanation of the hemolytic features.

# INFLUENCE OF VITAMIN B<sub>6</sub> AND PANTOTHENIC ACID ON GROWTH OF SARCOMA 180

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Studies from this laboratory<sup>1</sup> have shown that vitamins B<sub>1</sub> and B<sub>2</sub>, while having a profound effect on the nutritional state of the mouse, as indicated by caloric consumption and somatic growth, and thus an indirect effect on tumor growth, do not remedy deficiency of a factor the lack of which markedly retards tumor growth (sarcoma 180). The latter factor was relegated to the B<sub>6</sub>, or filtrate, fraction of the B complex. Morris and Lippincott<sup>2</sup> extended the studies on the B complex to pantothenic acid and spontaneous mammary carcinoma, and confirmed our finding. The present report, which is a study of the influence of vitamin B<sub>6</sub> and pantothenic acid on the growth of sarcoma 180, was begun when these substances became available in pure form.

## EXPERIMENTAL PROCEDURE

In experiment 1 synthetic diet 5, previously described,<sup>1</sup> which contains 25 parts vitamin-free casein, 63 parts starch, 4 parts hydrogenated cottonseed oil, 3 parts agar and 4 parts salt mixture, was used.<sup>3</sup> Nicotinic acid when administered was incorporated in 0.05 per cent concentration. Vitamins B<sub>1</sub>, B<sub>2</sub> and B<sub>6</sub> were injected parenterally, each in the amount of 0.01 mg. per mouse per day. In experiments 2 and 3 the diet consisted of 25 parts vitamin-free casein, 65 parts starch, 4 parts hydrogenated cottonseed oil, 3 parts agar and 2 parts salt mixture. The vitamin mixture described by Lippincott and Morris was added to this diet in the concentration recommended by the authors.<sup>4</sup> The complete diet used in experiments 2 and 3 differed from that used in experiment 1 essentially in the addition of choline hydrochloride and pantothenic acid. The experimental procedures for measuring

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From the Chemical Laboratory, Santa Barbara Cottage Hospital Research Institute.

1. Bischoff, F., and Long, M. L.: *Am. J. Cancer* **37**:54, 1939.

2. Morris, H. P., and Lippincott, S. W.: *J. Nat. Cancer Inst.* **2**:47, 1941.

3. The vitamin-free casein used was Borden's Labco vitamin-free casein, and the hydrogenated cottonseed oil was Crisco.

4. Lippincott, S. W., and Morris, H. P.: *J. Nat. Cancer Inst.* **2**:39, 1941.

caloric consumption, the technic of inoculation and the statistical analysis of the data on the tumors have been previously described.<sup>5</sup> In each experiment, 15 to 16 mice were used per diet group, 60 mice being used in experiment 2 and 48 mice in experiment 3. In experiment 1, a number of mice succumbed on the diets completely deficient in vitamins, and the data on the tumors are calculated for the surviving mice.

## RESULTS

The data in the accompanying table give the period during which the mice were maintained on each dietary regimen, the relation of the day of inoculation with tumor to the start of the regimen, the mean net change in somatic weight, the mean diameter of the tumors at the end of the

*Influence of Vitamin B<sub>6</sub> Deficiency and Pantothenic Acid Deficiency on the Growth of Sarcoma 180*

Experiment	Diet *	Days on Dietary Regimen	Day of Inoculation (on Regimen)	Mean Net Change in Somatic Weight, Gm.	Mean Diameter of Tumors, Mm.	Mean Consumption of Calories	
						Calories	Days
1	Synthetic diet 5.....	30	10	-9.2	5.9 ± 0.8	187	30
	Synthetic diet 5 + B <sub>6</sub> .....	30	10	-9.6	8.8 ± 0.6	208	30
	Synthetic diet 5 + B <sub>1</sub> , B <sub>2</sub> , nicotinic acid.....	30	10	-2.0	7.0 ± 0.9	304	30
	Synthetic diet 5 + B <sub>1</sub> , B <sub>2</sub> , B <sub>6</sub> , nicotinic acid.....	30	10	-2.3	12.2 ± 0.6	285	30
	Calf meal.....	30	10	+1.5	12.4 ± 0.7		
2	Calf meal.....	20	1	-0.3	19.0 ± 0.8	250	20
	Synthetic diet 10.....	20	1	+0.4	19.0 ± 1.0	242	20
	Synthetic diet 10 - B <sub>6</sub> .....	20	1	-2.0	12.5 ± 0.9	214	20
	Synthetic diet 10 - pantothenic acid.....	20	1	-1.7	18.0 ± 0.9	226	20
3	Synthetic diet 10.....	24	9	-1.6	15.7 ± 0.9	247	21
	Synthetic diet 10 - B <sub>6</sub> .....	24	9	-2.4	10.2 ± 1.0	221	21
	Synthetic diet 10 - pantothenic acid.....	24	9	-2.7	15.2 ± 0.9	246	21

\* Note that diet 5 is vitamin free, while diet 10 contains vitamins of the B complex.

dietary regimen and the mean caloric intake for the whole or the last period of the dietary regimen. The mean net change in somatic weight is calculated by subtracting the mean tumor weight from the mean net change in body weight. The statistic is the standard deviation of the mean.

In experiment 1 the addition of vitamin B<sub>6</sub> to the diet either with or without the other vitamins did not significantly influence the caloric intake but did significantly influence the rate of tumor growth. The effect is particularly striking in the series of mice which received vitamins B<sub>1</sub>, B<sub>2</sub> and nicotinic acid. The influence of vitamins B<sub>1</sub>, B<sub>2</sub> and nicotinic acid in increasing caloric intake and decreasing loss in body

5. Bischoff, F.; Long, M. L., and Maxwell, L. C.: *Am. J. Cancer* **24**:549, 1935.

weight without affecting tumor growth confirms the results of experiments previously reported.<sup>6</sup>

In experiments 2 and 3 the withdrawal of pantothenic acid from the diet was not followed by a significant reduction in caloric intake or in tumor growth but was followed by a slight reduction in body weight. Under the same conditions of experimentation the withdrawal of vitamin B<sub>6</sub> reduced tumor growth markedly.

In experiment 3 the mean weight of the thymus gland for 9 control mice was  $33.0 \pm 3$  mg.; that for 10 mice on the diet deficient in vitamin B<sub>6</sub> was  $22.6 \pm 2.2$  mg.

In experiment 1 all mice were returned to an adequate diet at the end of the thirty day period on the dietary regimen; in all cases the rate of growth of the tumors returned to the rate of the controls within a five day period; i. e., the mean diameter of the tumors of each group increased 4.1 to 5.0 mm. in five days. This procedure shows that the inherent growth characteristics of the tumor were not affected. In experiment 2 the tumors were weighed at the end of the period of the dietary regimen to ascertain whether the diameter and weight of the tumors were correlated to the degree ascertained for control tumors. In experiment 3 the tumors were reserved for histologic examination. The sections were given to the histologist (J. J. R.) in code. They were examined and classified as to: (1) tendencies toward invasion of fat or muscle; (2) tendencies toward invasion of fat; (3) necrosis; (4) site of necrosis, peripheral or central; (5) mitosis; (6) vascularity; (7) presence of odd mononuclear cells. Items 1, 2, 3, 5 and 6 were graded as to degree and the standard deviation of the mean was calculated for the mean result of each group. Items 4 and 7 were analyzed statistically by the all or none effect. The only significant finding was that the tendency to invade fat was significantly less for the tumors of the mice on the deficient diet. Since the tumors were growing at a greatly reduced rate, this finding would probably be without significance. Of interest is the negative finding for differences in degree of necrosis, mitosis and vascularity.

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6. Jones<sup>7</sup> has reported for the Jensen rat sarcoma that deprivation of vitamin B<sub>1</sub> apart from caloric restriction affected the future course of the tumor. This deduction is based on the regression of tumors of rats placed on an adequate diet after a period of B<sub>1</sub> deficiency with attending caloric restriction. In these experiments the controls were not placed on a diet restricted to the caloric intake of the rats which were on the diet deficient in vitamin B<sub>1</sub> and the deduction is therefore in our opinion unwarranted, as one of us, in collaboration with Long and Maxwell,<sup>5</sup> has previously reported that the characteristics of sarcoma 180 may be affected by marked caloric restriction on a diet otherwise adequate in specific nutrients. Bischoff and Long.<sup>1</sup>

7. Jones, J. L.: *Cancer Research* 2:697, 1942.

## COMMENT

The question always arises as to the significance of retardation in tumor growth (tumor diameter or weight). In the present experiments the addition of vitamin B<sub>6</sub> to a diet containing the other listed members of the B complex produced tumors which exceeded those of the groups deficient in vitamin B<sub>6</sub> by 200 to 250 per cent of weight.

In our experiments the longest period on the dietary regimen was thirty days. In working with a spontaneous tumor Morris and Lippincott were able to extend this period, but the difference in results obtained for these tumors through pantothenic acid is not entirely explained on this basis. The negative results in our experiments with pantothenic acid may be due to failure to deplete endogenous stores of pantothenic acid. The results for vitamin B<sub>6</sub> are so clearcut that they establish the essentiality of this substance for the maximum growth of sarcoma 180.

## SUMMARY

In three series of experiments comprising 122 Marsh-Buffalo mice, the maintenance on a synthetic diet containing vitamins of the B group other than B<sub>6</sub> produced a marked and significant decrease in the rate of tumor growth, which was corrected by the addition of vitamin B<sub>6</sub> without the caloric intake being significantly changed.

In two series of experiments running concurrently with the experiments with vitamin B<sub>6</sub> (31 additional mice), pantothenic acid deficiency was without influence on the rate of tumor growth.

In a single experiment comprising 30 mice, the addition of vitamin B<sub>6</sub> to a diet otherwise completely deficient in the B complex produced a significant increase in tumor growth.

# ANOMALIES OF THE AORTIC ARCH

PETER A. HERBUT, M.D.

PHILADELPHIA

Anatomic variations of the aortic arch in man pursue definite patterns. It is the purpose of this presentation to consider the development of the more common anomalies and to present illustrative cases of each type. The cases described are taken from the last 5,800 necropsies performed at the Jefferson Medical College Hospital from 1912 to 1942, inclusively.

## EMBRYOLOGY

The development of the normal aortic arch and its main branches has been described in detail by Congdon<sup>1</sup> and needs to be considered only briefly here. Of the six arches which develop on each side of the notochord, the first and second do not contribute to the aortic arch or to its immediate branches. The origin of each third arch becomes the common carotid artery. The proximal portion of the right fourth arch is incorporated in the right subclavian artery, while the entire left fourth arch remains as the definitive aortic arch. The fifth arches form no permanent vessels in the adult. The proximal portions of the sixth arches become the pulmonary arteries while the distal portion on the right side disappears and that on the left becomes the ductus arteriosus.

## ANOMALIES

Not always do the arches follow the specific normal courses just outlined. When, however, the complicated development and the delicate mechanisms which govern the evolution are considered, it is indeed surprising that deviations from the normal pattern are not more frequent. The major anomalies may be divided into five definite groups. In approximately the order of their frequency, the types are:

1. Coarctation of the aorta. This is perhaps the most familiar anomaly. It is a constriction of the aorta, either partial or complete, at, above or below the point at which the ductus arteriosus or the ligamentum arteriosum enters the aorta (fig. 1, *I*).

2. Patent ductus arteriosus. In this anomaly the ductus arteriosus, instead of obliterating as it should shortly after birth, remains patent throughout adult life (fig. 1, *II*).

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From the clinical laboratories, Jefferson Medical College Hospital.

1. Congdon, E. D.: *Contrib. Embryol.* 68:47, 1922.



3. Posterior right subclavian artery. Normally, the dorsal portion of the right fourth aortic arch, between the entrance of the seventh cervical intersegmental artery and the descending aorta, obliterates, and the anterior portion becomes the definitive right subclavian artery. Sometimes the anterior portion obliterates (fig. 1, *III*). The definitive right subclavian artery then originates from the last portion of the aortic arch, making its exit immediately distal to the left subclavian

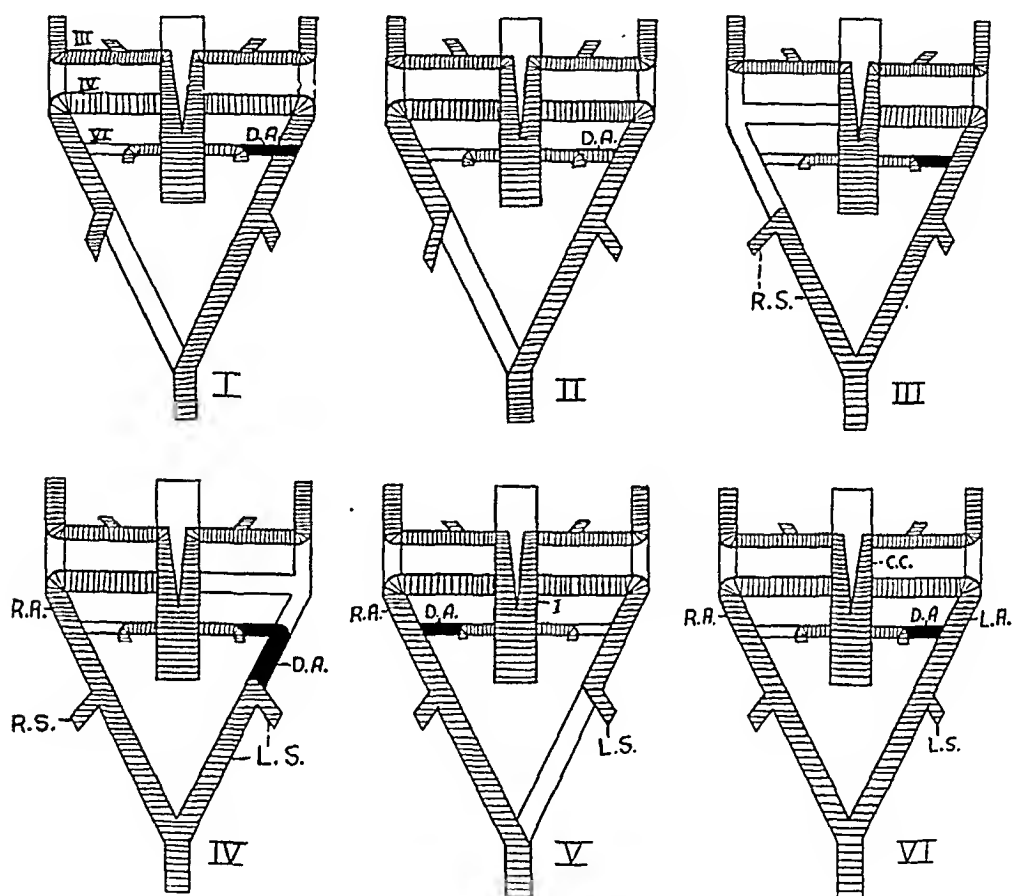


Fig. 1.—Diagrammatic sketch of the third, fourth and sixth arches. Shaded areas represent the definitive arteries; unshaded areas, the primitive portions that disappear. *I* represents coarctation of the aorta opposite an obliterated ductus arteriosus, *D.A.* *II*, shows a patent ductus arteriosus, *D.A.* *III*, shows a posterior right subclavian artery, *R.S.* *IV*, represents a right-sided aortic arch, *R.A.*, with a posterior left subclavian artery, *L.S.* *D.A.* is the obliterated ductus arteriosus; *R.S.*, the right subclavian artery. *V*, portrays a right-sided aortic arch, *R.A.*, with a left innominate artery, *I*, and an anterior left subclavian artery, *L.S.* The obliterated ductus arteriosus, *D.A.*, is on the right side. *VI*, shows a double aortic arch, *R.A.* (right aortic arch) and *L.A.* (left aortic arch). *D.A.* is the obliterated ductus arteriosus on the left side, *C.C.*, the common carotid arteries and *L.S.*, the left subclavian artery with a right subclavian artery symmetrically opposite.

artery. It pursues its course to the right side between the esophagus anteriorly and the vertebral bodies posteriorly.

4. Right-sided aortic arch. In this anomaly the right fourth aortic arch becomes the definitive aortic arch while the left one partially disappears. Most frequently that portion of the left arch between the aortic sac and the left seventh cervical intersegmental artery disappears (fig. 1, *IV*). The left subclavian artery then arises as the last branch of the right arch. Less frequently, the posterior portion of the left aortic arch between the seventh cervical intersegmental artery and the descending aorta disappears (fig. 1, *V*). In such instances the left subclavian arises from a left innominate artery.

5. Double aortic arch. This is the least common of the anomalies. Both the right and the left fourth aortic arch persist throughout life, forming a complete arterial collar about the trachea and esophagus (fig. 1, *VI*). There is no innominate artery. Both the right and the left common carotid and subclavian arteries arise independently and symmetrically from their respective arches.

#### CLINICAL CONSIDERATION WITH PRESENTATION OF CASES

*Coarctation.*—Although coarctation of the aorta is the most common anomaly, it is not of frequent occurrence. At this hospital there are recorded only 3 cases, giving a frequency of 0.05 per cent.

The symptoms of coarctation may be few and varied, or symptoms may be entirely absent.<sup>2</sup> When present, they are often those of hypertension,<sup>3</sup> which include precordial pain, headache, dizziness, epistaxis and vomiting, or those of cardiac failure. Physical examination reveals enlargement of the heart to the left. Systolic or diastolic murmurs, heard best at the base of the heart and transmitted to the neck or to the interscapular area, may be present. There is usually hypertension in the upper extremities and marked hypotension in the lower ones. When a collateral circulation is well established, the vessels originating proximal to the constriction may be felt and seen to pulsate. The diagnosis is confirmed by roentgenographic examination, showing erosions of the inferior margins and posterior portions of the ribs, or, when these do not appear, by intravenous injection of diodrast.<sup>4</sup>

The prognosis depends on the degree of stenosis, the extent to which a collateral circulation has been developed and the degree of hypertrophy

2. Abbot, M. E.: *Am. Heart J.* **3**:392, 1928. Evans, W.: *Quart. J. Med.* **26**:1, 1933.

3. Fray, W. B.: *Am. J. Roentgenol.* **24**:349, 1930. Railsback, O. C., and Dock, W.: *Radiology* **12**:58, 1929.

4. Blumenthal, S., and Davis, D. B.: *Am. J. Dis. Child.* **62**:1224, 1941. Grishman, A.; Steinbergh, M. F., and Sussman, M. L.: *Am. Heart J.* **21**:365, 1941.

of the left ventricle. Pregnancy is contraindicated.<sup>5</sup> The causes of death are myocardial failure, rupture of the ascending aorta, endocarditis and cerebral hemorrhage.<sup>6</sup>

CASE 1.<sup>7</sup>—A white man 26 years of age was admitted in shock. Four days previously, while carrying a timber at work, he experienced severe pain in the pit of the stomach. This necessitated rest in bed for two days. On returning to work, he experienced the same pain, but this time it was so severe that he collapsed. Prior to these episodes he was always in perfect health. Physical examination revealed pulsating vessels in the neck, the heart slightly enlarged transversely, a systolic murmur at the apex and blood pressure in the right arm averaging 130 systolic and 60 diastolic.

Necropsy showed constriction of the aortic arch directly opposite the ligamentum arteriosum. It measured 0.8 cm. in outside diameter, while the arch immediately above measured 1.3 cm. and the descending aorta immediately below 1.7 cm. The ascending aorta was 3.5 cm. in diameter at the base and 2 cm. at the level of the innominate artery. The main branches of the aortic arch and the internal mammary arteries were greatly dilated. The diameter of the innominate artery was 1.9 cm., that of the left common carotid artery 0.9 cm. and that of the left subclavian 1.3 cm. The ascending aorta contained at its base and posterior surface a ragged rupture, measuring 2.5 cm. in diameter. This was the starting point of a dissecting aneurysm which extended superiorly to the great vessels of the neck and inferiorly into the pericardial cavity. The latter contained 700 cc. of clotted and fluid blood. The coarctation was increased by the presence of a fibrous diaphragm in the intima opposite the external narrowing. Both ventricles were somewhat hypertrophied, the left measuring 2.5 cm. in thickness and the right 0.75 cm.

CASE 2.—A white woman aged 47 was admitted because of intermittent uterine bleeding of four months' duration. She had no complaints referable to the heart. Physical examination revealed a left-sided cardiac enlargement, a systolic murmur over the pulmonic area and at the base of the heart, and pulsating vessels in the neck. Fluoroscopic examination of the chest revealed no abnormality. A roentgenogram showed cardiac enlargement, a tortuous and dilated aorta and an aortic arch raised to the level of the suprasternal notch. The blood pressure varied from 120 to 150 systolic and from 60 to 100 diastolic. Hysterectomy was performed for carcinoma of the fundus of the uterus, after which the patient died of peritonitis.

Necropsy showed constriction of the aortic arch opposite the ligamentum arteriosum. The circumference of the aorta at this point was 5 cm., while that above was 9 cm. and that below 6 cm. The intima opposite the constriction disclosed a small ridge and dimple. The innominate, left common carotid and left subclavian arteries were definitely dilated. The heart showed relative left ventricular hypertrophy and some dilatation.

CASE 3.—A white boy 2 months of age was admitted with a history of cough for three days and fever for one day. He had been treated for thymic enlargement when 12 days old. Physical examination revealed rapid respiration, indrawing of

5. Mendelson, C. L.: *Am. J. Obst. & Gynec.* **39**:1014, 1941.

6. Baker, T. W., and Sheldon, W. D.: *Am. J. M. Sc.* **191**:626, 1936. Blackford, M. L.: *Arch. Int. Med.* **41**:702, 1928.

7. This case has been previously reported by Stewart, H. L., and Bellet, S.: *Am. Heart J.* **9**:533, 1934.

the suprasternal notch, marked venous pulsation in the neck, rales in the lungs and cardiac enlargement. A roentgenogram of the chest showed bronchopneumonia and a narrow upper mediastinal shadow.

Necropsy disclosed a heart weighing 74 Gm. The left ventricular wall measured 1 cm. in thickness. The foramen ovale was almost closed. The first part of the aorta and the innominate artery were dilated. The arch opposite the entrance of the ductus arteriosus was moderately constricted, measuring 1.2 cm. in circumference. The aorta above the constriction measured 2.5 cm. in circumference, while below the constriction it measured 2.0 cm. The lumen opposite the narrowing was patent. The ductus arteriosus is not mentioned in the protocol.

*Patent Ductus Arteriosus.*—Although much less frequently reported than coarctation of the aorta, this anomaly has recently gained much prominence because it is amenable to surgical treatment.

Early in life a retardation of growth may be the only presenting symptom; later, symptoms of cardiac failure or of other complications may be manifest.<sup>5</sup> Physical examination reveals an enlarged heart, a thrill over the precordium, a loud continuous murmur opposite the second and third interspaces, accentuation or reduplication of the second pulmonic sound and occasionally signs of aortic insufficiency. A roentgenogram shows a large heart, a prominent pulmonary conus and a congested pulmonary artery.<sup>9</sup> Intravenous roentgenography has so far been less successful in demonstrating a patent ductus.<sup>10</sup>

The ultimate prognosis, as a rule, is poor. By the age of 40 years, 71 per cent of the patients die from subacute bacterial endocarditis, congestive heart failure or rupture of the pulmonary artery.<sup>8b</sup>

The treatment is medical as long as the patient shows no evidence of retardation of physical development, cardiac hypertrophy, cardiac failure<sup>11</sup> or superimposed subacute bacterial endocarditis.<sup>12</sup> When, however, any of the complications named become manifest, excellent results may be obtained by surgical ligation of the patent ductus arteriosus.

CASE 4.—A white man 41 years of age was known to have congenital heart disease. He was otherwise healthy until twelve months previous to the illness in question. Then he began to have recurrent attacks of night sweats, general malaise and a low grade fever. Physical examination showed an enlarged heart, a diastolic and presystolic murmur at the apex, a palpable spleen, petechiae at both ankles, moderate anemia and a blood culture positive for *Streptococcus viridans*.

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8. (a) Gross, R. E.: J. A. M. A. **115**:1257, 1940. (b) Bullock, L. T.; Jones, J. C., and Dolley, F. S.: J. Pediat. **15**:786, 1939.

9. Eppinger, E. C., and Burwell, S. C.: J. A. M. A. **115**:1262, 1940. Gross.<sup>8a</sup>

10. Robb, G. P., and Steinberg, I.: J. A. M. A. **114**:474, 1940.

11. Hubbard, J. P.; Emerson, P. W., and Green, H.: New England J. Med. **221**:481, 1939. Shapiro, M. J.; Keys, A., and Violante, A.: Internat. Clin. **4**: 148, 1941.

12. Touroff, A. S. W., and Vesell, H.: J. Thoracic Surg. **10**:59, 1940. Touroff, A. S. W.; Vesell, H., and Chasoff, J.: J. A. M. A. **118**:890, 1942.

The electrocardiographic tracing was normal. A roentgenogram revealed "generalized intensification of the hilar and pulmonary vascular markings compatible with cardiac pathology." A diagnosis of patent ductus arteriosus with superimposed *Str. viridans* infection was made, and the patient was operated on. Uncontrollable hemorrhage occurred during the operation, and death ensued while the patient was on the operating table.

Necropsy showed a persistent ductus arteriosus, which measured 0.6 cm. in length along the superior border and 1.8 cm. along the inferior border. The outside diameter was 1.2 cm., while the inside was 0.6 cm. The ductus arose from the superior and anterior surface of the origin of the left pulmonary artery and entered the arch 0.8 cm. distal to the origin of the left subclavian artery. The entire ductus and the adjoining aorta were completely calcified. The wall of the ductus in its midlateral surface was lacerated over an area about 0.2 cm. in diameter, thus giving a direct communication between its lumen and the left pleural cavity. The intima of the ductus was roughened with atherosclerotic plaques. Its pulmonary side, in addition, contained numerous, large verrucae measuring as much as 0.6 cm. in diameter. Vegetations were also present in the main pulmonary artery. There were none in the arch of the aorta. The pulmonary artery was fusiformly dilated, measuring 4.8 cm. in diameter. Its wall was only 0.1 cm. thick. There was relative right-sided cardiac hypertrophy, the myocardium measuring 0.8 cm. in thickness. The lower lobe of the left lung contained several hemorrhagic infarcts measuring as much as 1.5 cm. in diameter. The spleen weighed 460 Gm.

CASE 5.—A woman 23 years of age was admitted, complaining of cough, fever and night sweats of two months' duration and of pain in the shoulder of two days' duration. She had been in poor health since the birth of a child six months previously. Physical examination revealed slight cyanosis of the lips, impaired percussion over the upper part of the chest on the left side, enlargement of the heart to the left, a thrill and a to and fro murmur over the pulmonic area, a slight fever and a blood culture positive for *Str. viridans*. The electrocardiogram was within normal limits. A roentgenogram showed transverse cardiac enlargement and an increase in pulmonary markings.

Necropsy showed a patent ductus arteriosus, from the lumen of which there protruded a large cauliflower-like vegetation, 2.5 cm. in diameter. The pulmonary artery was greatly dilated. The aortic arch was normal except for a large cone-shaped depression at the entrance of the ductus arteriosus. There were multiple red and gray pulmonary infarcts. The heart was uniformly hypertrophied, weighing 580 Gm.

CASE 6.—A white woman 24 years of age was admitted to the hospital for a cesarian section. She had a generally contracted pelvis and was known to have had heart disease for years. Physical examination revealed cardiac enlargement and an accentuated second pulmonic sound. An electrocardiogram showed right axis deviation. A roentgenogram disclosed a prominent pulmonary conus with calcification and right ventricular hypertrophy. Three days after the operation the patient became dyspneic and cyanotic and died.

At necropsy there was found a short, stubby, patent ductus arteriosus, measuring 0.4 cm. in length and 1.9 cm. in outside diameter. It was not calcified. It entered the left pulmonary artery 2.8 cm. distal to the origin of the main pulmonary vessel. Just to the left of the ductal entrance the origin of the left pulmonary artery contained a completely calcified saccular aneurysm, 2.5 cm. in diameter. The pulmonary artery was fusiformly dilated, measuring 3.2 cm. in diameter. The heart showed marked relative right-sided hypertrophy.

CASE 7.—A white woman 21 years of age was admitted, complaining of shortness of breath of three months' duration. During this time she intermittently resorted to rest in bed in order to carry on her daily activities. She was known to have had heart disease all her life. Physical examination revealed slight cyanosis of the lips and face, venous pulsation in the neck, cardiac enlargement to the left, a continuous murmur over the pulmonic area and an apical presystolic murmur transmitted to the axilla. An electrocardiogram showed marked right axis deviation. A roentgenogram revealed a prominent pulmonary conus, an enlarged heart and chronic congestive changes in both lungs. A diagnosis of patent ductus arteriosus was made and operation advised. The induction of anesthesia with nitrous oxide and oxygen was begun, but the patient died before the operation was started.

Necropsy disclosed a patent ductus arteriosus, 1 cm. long and 1.2 cm. in diameter. It entered the main pulmonary artery 4 cm. from the latter's base. There was no calcification, nor were there any vegetations. The pulmonary artery was fusiformly dilated, measuring 3.2 cm. in diameter. The heart weighed 500 Gm. It was globular in shape. The right ventricular wall was as thick as the left. There was considerable mitral fibrosis, with stenosis, but no evidence of active endocarditis.

*Posterior Right Subclavian Artery.*—Although occurring once in every 60 persons,<sup>13</sup> the posterior right subclavian artery rarely produces signs and symptoms. Dysphagia, inequality in radial pulses, pressure on the thoracic duct and trophic changes in the upper extremity have all been described.<sup>14</sup> The prognosis is good. Treatment usually is unnecessary, but when symptoms do develop, there is always the remote possibility of surgical ligation of the vessel.<sup>15</sup>

CASE 8.—A white boy 3 weeks old was admitted with a history of failure to gain weight and of cyanosis when straining or crying. Physical examination showed an undernourished infant with marked cyanosis. The cardiac rate was rapid, and a systolic murmur was heard over the sternum at the second intercostal space. A roentgenogram revealed a cervical rib on the right side and fused fourth and fifth ribs posteriorly.

Necropsy revealed an enlarged heart. There was only one atrium, and it opened into the left ventricle. The right ventricle was very small, and the pulmonary artery was markedly hypoplastic. The upper portion of the interventricular septum was defective. There was no ductus arteriosus. The aortic arch was normal in position. The innominate artery was absent, having been replaced by the right common carotid artery. The remaining branches were the left common carotid, the left subclavian and the right subclavian. The latter arose opposite the left subclavian artery and then proceeded to the left of and posterior to the esophagus to reach its usual destination on the right side (fig. 2). The esophagus was grooved by the vessel. The right recurrent laryngeal nerve was replaced by branches passing directly to the larynx.

CASE 9.—A girl 8 hours old was admitted because of cyanosis and noisy breathing. Physical examination showed marked cyanosis, laryngeal stridor and slow

13. Goldbloom, A. A.: Surg., Gynec. & Obst. **34**:378, 1922.

14. Anson, B. J.: Surg., Gynec. & Obst. **62**:708, 1936.

15. Cairney, J.: J. Anat. **59**:265, 1924-1925.

respirations. The heart sounds were regular. Tracheotomy was performed because of the stridor, but the infant died.

Necropsy showed a normal aortic arch. The main branches from right to left were the right common carotid, the left common carotid, the left subclavian and the right subclavian. The latter crossed the midline between the esophagus and the vertebral bodies. The ductus arteriosus and the foramen ovale were patent. There were no other cardiac defects. Examination of the head revealed no intracranial hemorrhage. The right recurrent laryngeal nerve was replaced by branches going directly to the larynx. The infant also possessed a cleft palate, an extra finger, a double vagina and an imperfect bicornuate uterus.

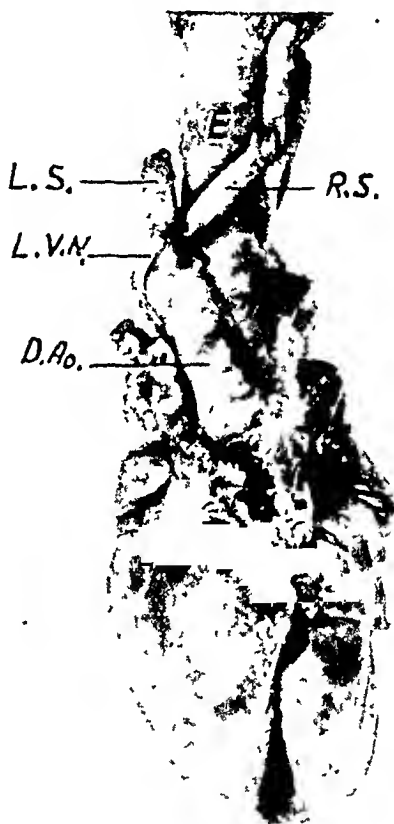


Fig. 2.—Posterior aspect showing the right subclavian artery, *R.S.*, arising from the arch opposite the left subclavian artery, *L.S.* *D.Ao.* is the descending aorta, *L.V.N.* the left vagus nerve and *E*, the esophagus.

*Right-Sided Aortic Arch.*—The right-sided aortic arch is often asymptomatic until later life, when dilatation and tortuosity of the aorta may cause pressure on the upper mediastinal structures, with resulting dysphagia, dyspnea and laryngeal paralysis.<sup>16</sup> Except for suprasternal pulsation, physical examination usually discloses no abnormality.<sup>17</sup>

16. (a) Sprague, H. B.; Ernlund, C. H., and Albright, F.: *New England J. Med.* **209**:679, 1933. (b) Bedford, D. E., and Parkinson, J.: *Brit J. Radiol* **9**: 776, 1936

17. Brigham, R. O.: *Ohio State M. J.* **18**:484, 1922.

Bronchoscopic and esophagoscopy inspection reveal a pulsating tumor mass on the right side.<sup>18</sup> Roentgenographically there are widening of the upper part of the mediastinal shadow to the right, pulsation of the aorta in the same location, concave depression of the right and posterior surface of a barium sulfate-filled esophagus and, on intravenous injection of diodrast, presence of the shadow of the arch of the aorta to the right of the trachea.<sup>19</sup> The prognosis is good. Treatment is usually unnecessary. In patients presenting symptoms of pressure caused by obliteration of the ductus arteriosus, the obstruction may possibly be relieved by surgical means.<sup>20</sup>

CASE 10.—A white woman 63 years of age was known to have had diabetes for years and had no symptoms referable to the heart or the upper part of the mediastinum. The trachea was in the midline, and the blood pressure varied from 130 to 165 systolic and from 60 to 120 diastolic.

Necropsy revealed an aortic arch passing to the right of the trachea and esophagus and then crossing to the left side at the level of the sixth dorsal vertebra (fig. 3). The left common carotid artery was the first branch. It crossed anterior to the trachea. The next branches were the right common carotid and the right subclavian. There was a persistent posterior aortic root, 2 cm. long, which crossed behind the esophagus. From its tip arose the left subclavian artery. The ligamentum arteriosum measured 2.4 by 0.3 cm. It connected the pulmonary artery with the tip of the left aortic root. The right and the left vagus and the recurrent laryngeal nerves were disposed in a symmetric manner about the right aortic arch and the ligamentum arteriosum, respectively. The anterior portion of the left aortic arch was entirely absent.

*Double Aortic Arch.*—The double aortic arch is the least common of the anomalies. In 1936 Blincoe, Lowance and Venable<sup>21</sup> collected from the literature a total of 40 cases. Although adults with this condition as a rule are asymptomatic, infants present highly characteristic signs and symptoms. As outlined by Wolman,<sup>22</sup> these consist of "stridulous breathing, chronic cough, respiratory distress while feeding, failure to thrive, susceptibility to pneumonia, head retraction and malnutrition." Physical examination as a rule reveals nothing characteristic. Bronchoscopic inspection may or may not disclose a constriction of the trachea.<sup>23</sup> In infants, a roentgenogram shows a widening of the superior mediastinum, interpreted almost invariably as a persistent or enlarged thymus. It has been stated that a barium sulfate-filled

18. Garland, L. H.: *Am. J. Roentgenol.* **39**:713, 1938.

19 (a) Metzger, H. N., and Ostrum, H.: *Am. J. Digest. Dis. & Nutrition* **6**:32, 1939. (b) Roche, U. J.; Steinberg, I., and Robb, G. P.: *Arch. Int. Med.* **67**:995, 1941.

20. Jex-Blake, A. J.: *Lancet* **2**:542, 1926. Sprague and others.<sup>16a</sup>

21. Blincoe, H.; Lowance, M. I., and Venable, J.: *Anat. Rec.* **66**:505, 1936.

22. Wolman, I. J.: *J. Pediat.* **14**:527, 1939.

23. (a) Hermann, W. W.: *Arch. Path.* **6**:419, 1928. (b) Schall, L. A., and Johnson, L. G.: *Ann. Otol., Rhin. & Laryng.* **49**:1055, 1940.



esophagus shows a uniform constriction at the level of the arch of the aorta, although, as far as I know, this has really never been demonstrated.

Thus far,<sup>24</sup> treatment of patients presenting symptoms has been entirely palliative and without success. If, however, a diagnosis can be made relatively early, it may be possible to sever the arterial constriction surgically.<sup>25</sup> This has never been attempted.

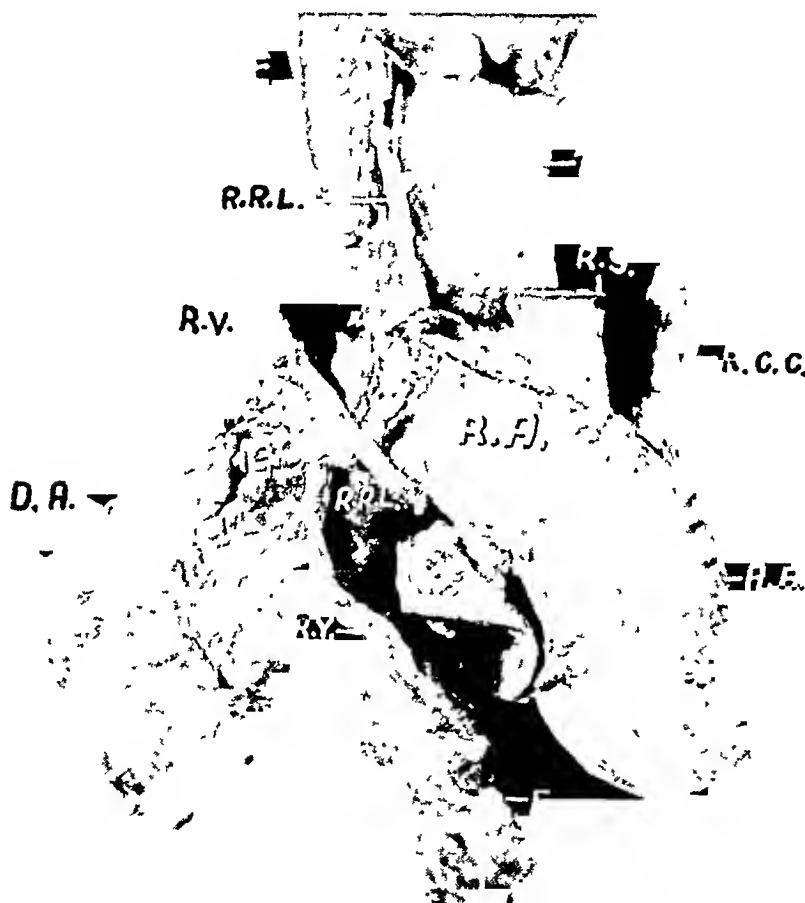


Fig. 3.—Right lateral view showing a right-sided aortic arch, *R.A.* *A.A.* is the ascending aorta, *D.A.* the descending aorta, *R.C.C.* the right common carotid artery, *R.S.* the right subclavian artery, *R.P.* the right vagus nerve, and *R.R.L.* and *R.R.L.*' the right recurrent laryngeal nerve, *E* the esophagus and *T* the trachea.

CASE 11.—A Negro man 39 years of age was admitted with symptoms and signs of Addison's disease. On only one occasion, ten months previous to admis-

24. Roche and others.<sup>19b</sup> Wolman.<sup>22</sup> Schall and Johnson.<sup>23b</sup>

25. (a) Herbut, P. A., and Smith, T. T.: *Arch. Otolaryng.* **37**:558, 1943.  
(b) Wolman.<sup>22</sup>

sion, when he experienced a weak feeling, did he complain of dyspnea. This disappeared with rest in bed. Physical and electrocardiographic examination of the heart indicated that it was normal. A roentgenogram of the chest was not made.

Necropsy showed a complete double aortic arch originating from the upper end of the ascending aorta, encircling the trachea and esophagus and ending in the descending aorta posterior to the esophagus. The right arch measured 1.8 cm. and the left 1.2 cm. in diameter. The main branches arising symmetrically from their corresponding arches were the right and the left common carotid artery and the right and the left subclavian artery. The ligamentum arteriosum was present in its usual position on the left side. Both the right and the left vagus and the recurrent laryngeal nerves were symmetrically disposed around their respective arches. There was no evident constriction of the esophagus, nor was there ulceration of its mucosa. There was tuberculous involvement of each adrenal gland.

CASE 12.—A report of this case was published in the April issue of the *Archives of Otolaryngology*.<sup>25a</sup> A white girl 2½ months of age was admitted with a history of attacks of dyspnea since birth. More recently these attacks had been associated with cyanosis and were particularly severe during feeding. Physical examination gave essentially negative results, as did roentgen examination of the chest. In the hospital, several attacks of dyspnea were observed, during which her head was turned to the left, her neck extended and her head flexed. Bronchoscopic examination was attempted but, because of the dyspnea, could not be carried out. Tracheotomy did not relieve the respiratory obstruction, and the child died five days after admission.

Necropsy disclosed a double aortic arch forming a complete arterial collar about the trachea and esophagus (fig. 4). The right arch measured 1.0 cm. and the left 0.5 cm. in diameter. As in the preceding case, the larger vessels arose symmetrically from their corresponding arches. The first branches were the right and the left common carotid, followed by the right and the left subclavian artery. The ductus arteriosus measured 1 cm. in length and 0.4 cm. in diameter. All the vessels were patent. The esophagus was definitely constricted, but its mucosa was not ulcerated. Compression of the trachea could not be satisfactorily demonstrated at postmortem examination. There was no pneumonia.

#### COMMENT

The disposition of the recurrent laryngeal nerves is worthy of mention. They are normal in cases of coarctation of the aorta and in cases of patent ductus arteriosus. In cases of posterior right subclavian artery the right recurrent laryngeal nerve is usually replaced by branches passing directly from the vagus nerve to the larynx. Occasionally, it winds around the inferior thyroid artery, while at other times it envelops the right vertebral artery.<sup>15</sup> In cases of double and cases of right-sided aortic arch the right recurrent laryngeal nerve passes around the arch instead of around the right subclavian artery. The position of the left recurrent laryngeal nerve varies. It is normal when there is also a left aortic arch or when the ductus arteriosus is on the left side, joining the pulmonary artery with a persistent left aortic root. When there is a

left innominate artery and the ligamentum arteriosum is on the right side, the left recurrent laryngeal nerve winds around the left subclavian artery.<sup>26</sup>

A word should also be added about the intravenous use of diodrast. The technic as perfected by Robb and Steinberg<sup>10</sup> appears to be simple and certainly gives excellent results. Special apparatus, however, is needed, and the operator must be highly skilled. It therefore is not



Fig. 4.—Posterior view showing the left aortic arch, *L.A.A.*, and the right aortic arch, *R.A.A.*, uniting to form the descending aorta, *D.Ao.* *L.S.* is the left subclavian artery, *R.S.* the right subclavian artery, *D.A.* the ductus arteriosus and *E* the esophagus.

practical for the ordinary hospital. The injection of diodrast in itself is not without danger. Robb and Steinberg<sup>10</sup> have made 486 injections in 233 patients, many of whom were seriously ill, and have seen no serious consequences. Occasionally, however, the intravenous use of diodrast in excretory urography has produced death. Nine such cases

26. Sprong, D. H., and Cutler, N. L.: *Anat. Rec.* 45:365, 1930.

have been reported in the literature.<sup>27</sup> Doubtless, there are many similar instances which have not been reported. It therefore seems reasonable to suggest that when a positive diagnosis can be made by less dangerous means, such as roentgen study of a barium sulfate-filled esophagus, esophagoscopy and bronchoscopy, intravenous injection of diodrast should not be resorted to. If it is used, the patient should first be tested for sensitivity to iodine.

#### SUMMARY

Anomalies of the aortic arch may be divided into five separate groups. In approximately the order of frequency the types are: coarctation of the aorta, patent ductus arteriosus, posterior right subclavian artery, right-sided aortic arch and double aortic arch. All of these are easily explained embryologically. For an antemortem diagnosis, bronchoscopic, esophagoscopy and roentgenographic studies are indispensable. Surgical intervention gives excellent results in cases of patent ductus arteriosus. The other anomalies are less amenable to treatment. This is particularly true of a double aortic arch in an infant. All infants presenting symptoms of this anomaly have died of strangulation.

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27. Goldburgh, H. L., and Baer, S.: *J. A. M. A.* **118**:1051, 1942.

## Case Reports

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### DIFFUSE METAPLASTIC GASTRITIS IN A PATIENT WITH PROLONGED CACHEXIA AND MACROCYTIC ANEMIA

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In man and higher mammals a so-called law of specificity of tissue growth postulates that the cells composing a given structure in the fully developed organism are capable of reproducing only identical morphologic and functional types. Exceptions to this rule are encountered in pathologic states and relate principally to the epithelial lining surfaces and the supporting connective tissues of the body. Morbid alterations of epithelial growth in particular have been observed in various organs subject to prolonged inflammation or deprived of adequate nutrition. Thus the tall columnar epithelial cells lining the bronchial tubes not uncommonly acquire a stratified squamous composition under the local stimulus of some chronic bacterial, mechanical or chemical irritation or during the course of certain long-standing dietary deficiencies. Less often, squamous metaplasia is found in the mucosa of the urinary bladder, renal pelvis, ureter, urethra, accessory nasal sinuses, gallbladder, prostatic gland, pancreatic ducts, portions of the gastroenteric canal and elsewhere. When these variants become established in any adult tissue, the process appears largely irreversible, replacement and growth of the altered tissue taking place by reproduction of the acquired type of epithelial cell. It is noteworthy, however, that in certain neoplasms, presumably arising from metaplastic epithelium, both the original and the acquired form of epithelial cell may be observed in the same tumor. Two distinct types of epithelial metamorphosis have been described as involving the mucosal coat of the gastroenteric tract. One of these undoubtedly illustrates heterotopia rather than true metaplasia and proceeds unrelated to local inflammatory or to general deficiency states. This form of epithelium is well developed, functionally active and of wide distribution; typical examples are: bits of gastric mucosa within a diverticulum of Meckel's type, scattered nests of pancreatic glands within the stomach or throughout the small intestine, portions of gastric mucosa indenting the lining coat of the lower part of the esophagus and zones of esophageal mucosal epithelium interpolated between the gastric glands of the cardiac end of the stomach. The other type of epithelial mutation represents true metaplasia. It has been infrequently observed in the colon and rectum and is extremely rare in the gastric mucosa, where it follows severe local inflammatory reactions or complicates prolonged dietary deficiencies. This transformation of the glandular epithelium tends toward a frank squamous cell type but is quite restricted

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in its degree of development, extent and distribution; seldom does it encompass more than a tiny segment of the organ involved.

Diffuse areas of squamous metaplasia, regardless of the causative factors, have hitherto not been described in the gastric mucosa as far as can be determined by an examination of the literature. Likewise, nothing appears to be known of such a condition elsewhere in the intestinal tract. The case under consideration is particularly arresting in that the tissue showing metaplastic changes comprised almost two thirds of the entire lining surface of an unusually small stomach. The nature and the extent of this condition raise several points of interest relating to gastric physiology and general metabolism.

#### REPORT OF A CASE

A Negro woman 52 years of age was first admitted to the Cincinnati General Hospital Jan. 18, 1935, in a profoundly weak and stuporous condition. It was learned that she had been suffering from intermittent attacks of vomiting and midabdominal pain for several years. These symptoms had increased markedly in severity during the preceding month, forcing her to remain in bed. She stated that in recent weeks the vomiting began immediately after eating food of any kind, or even after drinking water, and was followed by pain, usually localized around the umbilicus. Hematemesis was never present. She was accustomed to take mild laxatives daily. Her loss of weight in the past month was reckoned at 5 to 10 pounds (2.3 to 4.5 Kg.). For two weeks she had also suffered from frequency of urination and dysuria with the passage of some blood. The menopause had occurred twelve years previously. The patient presented no significant positive physical findings other than marked emaciation. Urine obtained by catheter was loaded with leukocytes, and the benzidine test was positive. The blood urea nitrogen amounted to 14 mg., and the blood sugar during fasting to 57 mg., per hundred cubic centimeters. An erythrocyte count made a week after admission was 2,400,000; no remarks regarding the appearance of the cells were recorded. Under a regimen of frequent ingestion of easily assimilable foods, abundant intake of fluids and other supportive measures, the vomiting and abdominal pain subsided; the patient gained strength slowly and showed progressive improvement in general condition but continued to complain of various unrelated and transient symptoms. An intravenous pyelogram made February 15 disclosed marked hydronephrosis on the right. The involved kidney was quite mobile, descending to the level of the first sacral segment from the upper level of the third lumbar body. On the assumption that much of her vague complaints could be relieved by correcting the nephroptosis on the right, the patient was transferred to the surgical service. February 21, nephropexy was performed, with the patient under local anesthesia, and since there were no untoward postoperative incidents, she was discharged. March 16, considerably improved, with instructions to return one month later for further observation. There is no record that a Kahn or a Wassermann test was made on the blood during this period of hospitalization. The patient failed to keep her appointment and was not seen again for almost nineteen months.

Oct. 2, 1936, the patient was again brought into the hospital in a semicomatose state, unable to give a coherent account of her condition. On general supportive measures she improved sufficiently to tell her history in some detail ten days later. She stated that her present trouble began six months previously following a day of overexertion at heavy manual labor. The following day she complained of fatigue and vomited several times. She recalled that these vomiting spells had

occurred once every two or three weeks for about three years but could not be attributed to any reasonable cause. During the last six months the vomiting began to occur more frequently. About five months before admission she noted some numbness in her fingers and toes and thereafter perceived a more or less continual tingling sensation in these members. This continued throughout the ensuing months, and about six weeks before hospitalization exertional dyspnea and unsteadiness of gait began to appear. On questioning it was learned that her tongue had been sore for about one year; this she attributed to eating coarse foods. All her teeth had been extracted some time before. Three weeks preceding admission severe watery diarrhea started suddenly, and she passed fifteen to twenty stools daily, which occasionally were tinged with blood. Shortly after this began, she started coughing and expectorated considerable mucopurulent sputum. Feeling extremely weak and apprehensive, she asked to be taken to the hospital. After detailed questioning about her past history, she remembered that she had had typhoid fever as a youngster, and also pneumonia on one occasion, though the dates could not be recalled. Her dietary habits had been irregular, probably for most of her life, and the choice of foods extremely limited. During the past several years she had had \$2.50 a week to spend on food; an undetermined portion of this sum was used for cigarets and other items. She maintained, however, that despite her frugal diet, her appetite remained good for foods that she could not afford. The patient had been married but to her knowledge was never pregnant. She learned in 1925 that she had acquired syphilis from her husband. The antisyphilitic treatment recommended by a physician was refused. For a number of years she had subsisted entirely on charity and had lived in squalid surroundings. She drank any type of alcoholic liquor that was available but was apparently limited in its intemperate use by financial circumstances.

The day following her second admission the essential findings were as follows: The temperature was 97 F.; the pulse rate, 89; the respiratory rate, 30; the blood pressure, 85 systolic and 55 diastolic. Emaciation was pronounced. The mucous membranes were pale. The edges and posterior third of her tongue were smooth and the papillae atrophied. Many coarse rales were heard over the entire back. Both knee jerks were absent. Some diminution of vibratory sense was present in the lower parts of both legs, more marked on the right side. Roentgen examination revealed discrete and confluent areas of bronchopneumonia throughout the lower portions of both lungs, most marked at the right base.

The Kahn test of the blood was positive (3 plus). The icteric index was 11. The blood chloride amounted to 524 mg., the blood urea nitrogen to 48 mg. and the blood sugar to 74 mg. per hundred cubic centimeters. The carbon dioxide-combining power was 46 volumes per cent. Stool cultures were negative for typhoid and dysentery bacilli. A dextrose tolerance test gave results within the normal limits. The hemoglobin content was 8 Gm.; the erythrocyte count, 1,950,000; the leukocyte count, 8,950, with polymorphonuclear neutrophils 88 per cent. The red blood cells were enlarged, distorted and well filled with hemoglobin; no immature forms were noted. The specific gravity of the urine was 1.015; there was albumin (plus 1); the leukocytes numbered 50 per low power field; a few erythrocytes and some epithelial cells were found. Gastric analysis after administration of histamine showed no free hydrochloric acid.

In view of the severe macrocytic anemia associated with complete gastric achlorhydria, sore tongue, tingling sensations, numbness and evidence of damage

to the vibratory sense in the extremities, a diagnosis of probable pernicious anemia was made. Sprue was also considered, although examinations of the stools lent little support to this possibility. Supportive treatment was instituted for the bronchopneumonia and the unexplained diarrhea, which rapidly subsided. The patient was given several transfusions of whole blood and large intramuscular injections of liver extract, and in an attempt to restore the weight she had lost, she was placed on a high caloric diet including brewer's yeast. Despite the intensive liver therapy, the reticulocyte count never exceeded 2 to 3 per cent. After two weeks of hospitalization, a cutaneous lesion developed over the left side of the forehead, which was diagnosed as herpes zoster of the ophthalmic branch of the fifth nerve. This subsided uneventfully. The blood urea nitrogen on October 19 amounted to 20 mg. per hundred cubic centimeters; the total serum protein amounted to 8.7 Gm., the serum albumin to 4.6 Gm. and the serum globulin to

*Blood Counts and Weight*

Date	Hemo- globin, Gm.	Red Blood Cell Count	White Blood Cell Count	Poly- mor- pho- nu- clears	Lym- pho- cytes	Mono- cytes	Reti- culo- cytes	Body Weight, Lb.	Comment
1/26/35	...	2,400,000	.....	..	..	...	...	....	
10/ 2/36	8.0	1,950,000	8,950	88	10	2.0	0.7	....	Moderate poikilo- cytosis; red blood cells large and well filled with hemo- globin; no nucle- ated forms noted. Average corpus- cular volume 121 cubic microns. Average corpuscu- lar hemoglobin 47 micromicrograms
10/ 9/36	10.5	2,200,000	10,000	..	..	...	0.5	73.5	
10/12/36	8.0	2,200,000	9,500	65	35	...	0.8	....	
10/19/36	7.2	2,000,000	8,100	74	24	2.0	3.0	69.0	
10/27/36	9.0	1,880,000	.....	..	..	...	1.9	....	
11/ 1/36	9.0	2,660,000	.....	..	..	...	...	70.0	
11/16/36	9.6	3,650,000	.....	..	..	...	...	....	
11/30/36	9.4	4,200,000	13,200	70	29	1.0	...	....	
12/ 3/36	...	.....	.....	..	..	...	...	70.75	
12/27/36	...	4,350,000	26,350	92	5	3.0	...	....	
1/ 5/37	10.0	4,040,000	12,200	79	11	10.0	...	....	
1/29/37	10.0	5,370,000	.....	..	..	...	...	65.0	Sedimentation rate 58 mm. Hematocrit read- ing 32 per cent
2/19/37	11.5	3,540,000	8,960	..	..	...	...	....	
3/19/37	9.7	3,850,000	.....	..	..	...	...	....	
4/ 8/37	...	.....	.....	..	..	...	...	78.75	
8/11/39	10.5	3,650,000	.....	..	..	...	...	92.0	
9/15/39	....	.....	.....	..	..	...	...	86.5	
11/24/39	....	.....	.....	..	..	...	...	93.75	
10/ 6/40	5.0	1,550,000	4,450	..	..	...	...	....	

4.1 Gm. per hundred cubic centimeters. The following day a Wassermann test of the spinal fluid and a colloidal gold test were reported negative. The blood picture improved steadily, and November 1 the hemoglobin was 9.9 Gm. and the erythrocyte count 2,660,000. Two months later the hemoglobin had reached 10 Gm. and the red blood cell count 4,040,000 (table). Morphologically, the erythrocytes now apparently contained a normal amount of hemoglobin. The basal metabolic rate December 29 was +13 per cent. Roentgenograms of the gastroenteric tract made the following day showed the stomach to be small and contracted, lying almost entirely in the left upper abdominal quadrant. Its walls were smooth and showed no active peristaltic waves. A concentric tubular deformity of the pyloric end was constantly noted (fig. 1). The duodenal bulb was small and led into a dilated second and third portion of the duodenum. When the patient was in an extreme Trendelenburg position, the barium sulfate regurgitated into the esophagus through the cardiac sphincter. The rigidity of the stomach suggested linitis plastica. At the end of twenty-four hours there was 50 per cent gastric retention. A gastroscopic examination Jan. 4, 1937



revealed that the mucosa was pale and the rugae everywhere absent; the pylorus and the antrum were not visualized. The impression at this time was that of atrophic gastritis.

At this time doubts about the original clinical diagnosis were expressed. Because of the absence of immature red cells in the peripheral blood and the failure of the reticulocytes to respond to liver therapy, and in view of the old history of acquired syphilis, the strongly positive Wassermann reaction of the blood and the unusual appearance of the stomach when studied roentgenologically



Fig. 1.—A roentgen film taken immediately after a fluoroscopic examination of the stomach. The funnel-shaped narrowing of the stomach appears more prominent over its lower half.

and gastroscopically, a diagnosis of gastric syphilis was held probable. The gastric content, examined repeatedly after injections of histamine, continued to show absence of free hydrochloric acid. The patient was given several intramuscular injections of bismuth subsalicylate and large intravenous doses of sodium iodide. A course of nine intravenous injections was begun with an initial dose of 0.3 Gm. of neoarsphenamine followed by four more of 0.6 Gm. each at weekly intervals; for the last four injections the dose was increased to 0.9 Gm. After the pneumonia had subsided the patient remained afebrile with minor

exceptions for the duration of her hospital course. She was discharged, April 8, as improved, weighing  $78\frac{3}{4}$  pounds (35.8 Kg.), after seven months' hospitalization and was advised to return for further treatment and observation.

She was seen again June 18 and said that her appetite was good and that she felt fine. A gastroenteric series June 22 showed no gastric residue at the end of twenty-four hours but otherwise showed conditions similar to those at the previous examination. The patient neglected to return for observation and was lost sight of for a period of two years. July 3, 1939, she reappeared unexpectedly, saying that she had been quite well until three months before, when she began to lose appetite and to vomit occasionally. She also felt that she was getting weaker and said that she had lost 10 pounds (4.5 Kg.). Her best weight had been 105 pounds (47.6 Kg.), sometime in May 1939. There had been some numbness and tingling of the hands and feet during the past year. At the time she weighed 95 pounds (43 Kg.) and despite complaints her general condition showed considerable improvement over that two years before. Her mucous membranes were slightly pale. Palliative measures were advised for her complaints, and she was told to return a week later. A gastroenteric series was made June 11, which yielded findings essentially the same as those previously noted. Reexamination with the gastroscope September 22 again gave the impression of atrophic gastritis, but the possibility of diffuse fibrosis of the gastric mucosa was considered. When seen again, November 24, she still complained of numbness and tingling of her hands and feet but was otherwise symptom free. Her weight was  $93\frac{3}{4}$  pounds (42.5 Kg.). She was to return in two months but did not appear until Oct. 6, 1940, ten months later.

On that date she was brought to the hospital by ambulance at 1:51 p. m., critically ill and unable to respond to questioning. A friend who accompanied her volunteered the information that she had been steadily growing weaker and during the past few weeks had often complained of abdominal pain. For several days preceding admission she had been unable to void, and weakness obliged her to remain in bed. On admission the bladder was catheterized and 700 cc. of bloody urine withdrawn. The patient was critically ill, emaciated and slightly dehydrated. The temperature was 98 F., the pulse rate 88, the respiratory rate 20 and the blood pressure 80 systolic and 50 diastolic. There was generalized abdominal tenderness to palpation. The mucous membranes were extremely pale. The hemoglobin content was 5 Gm., the red blood cell count 1,550,000 and the leukocyte count 4,450. The blood urea nitrogen amounted to 75 mg. per hundred cubic centimeters. The carbon dioxide-combining power was 30 volumes per cent. An immediate transfusion of 250 cc. of whole blood was given. Her condition became critical, and she died at 3:55 p. m. the following day.

The final impression was one of marked cachexia, probably due to gastric carcinoma; uremia; syphilis (?) of the stomach; marked anemia.

*Autopsy* (fifty hours after death).—The body, measuring 160 cm. in length, was that of an extremely poorly nourished mulatto woman, appearing approximately the stated age of 57 years. Rigor mortis was well developed in the muscles of the extremities, neck and jaws. There were no external deformities. There was moderate pitting edema of the feet and legs, extending to both knees. The inelastic smooth skin was free from eruptions and scars. The gums and the mucous membrane were pale pink, and the jaws edentulous. Both corneas were cloudy. The panniculus over both the thorax and the abdomen was extremely thin and light yellow. The underlying dark red musculature was thin and poorly developed. The peritoneal cavity contained a moderate amount of thin yellow

fibrinopurulent exudate collected in small areas about the loops of the small intestine. The mesenteric lymph nodes were slightly enlarged. The pleurae were glistening gray except where covered by thin fibrous adhesions. The lining of the pericardial sac was smooth, gray and glistening. There were no external abnormalities about the neck, and the organs here were not removed. The thoracic portion of the esophagus appeared slightly dilated and was filled with light yellow watery material.

The small, light red heart weighed 210 Gm. The endocardial lining was smooth and translucent, and the valve leaflets were thin and free from scarring. Sections through the interventricular septum and the left ventricular wall showed a soft, flabby, pale red musculature. The coronary arteries were thick and the intimal surfaces covered by irregular patches of opaque, brittle, yellow material. The inelastic, nontortuous abdominal aorta presented many irregular raised opaque yellow intimal plaques. A few of these were ulcerated.

The lungs, right and left, weighed 700 and 450 Gm., respectively. All the lobes were rather poorly aerated and exuded moderate amounts of dark red watery fluid on pressure. No areas of consolidation were present. The hilar lymph nodes were slightly enlarged and presented black pigment.

The reddish brown spleen weighed 175 Gm.; it was surrounded by thick fibrous adhesions which were broken with difficulty. The splenic substance was dull pink and friable, with moderately accentuated follicular markings.

The pale red liver weighed 1,400 Gm. and was covered by a thin translucent capsule except for a few small thickened areas. The liver tissue was pale reddish brown, soft, friable, slightly greasy. The gallbladder was filled with approximately 75 cc. of greenish black bile and had a dark greenish black velvety mucosal lining. The biliary passages were patent.

The pancreas appeared normal. The stomach was extremely small, measuring 26 cm. along the greater curvature from the esophageal junction to the pyloric ring (fig. 2). Its outline was fairly regular, although the reduction in size appeared slightly more pronounced over the lower half. While the caliber of the pylorus was uniformly reduced, there was no evidence of any obstruction. The wall was markedly thickened and cut with a gritty sound. The rugae were everywhere flattened and rarely visible, the mucosal coat dull gray and lusterless. The valvulae conniventes of the duodenum and jejunum were distinct. The esophagus appeared slightly dilated, its mucosa smooth and gray. No abnormalities were present at the cardioesophageal junction. The mucosa and wall of the colon showed no noteworthy changes. The peritoneum covering many of the lower loops of small intestine was thick, lusterless and coated by a small amount of thick yellow fibrinous exudate.

The two equal-sized kidneys together weighed 225 Gm. Their stripped surfaces were pale grayish red and nongranular. Coronal sections showed cortices and medullae quite well defined, with occasional slender gray streaks extending from the medullary tips well into the cortex. The pelves appeared normal in size, and their mucosal lining was dusky reddish gray. The ureters possessed smooth thin glistening white linings.

The urinary bladder contained approximately 500 cc. of bright red urine. The bladder wall was thickened, dark red and extremely friable; the mucosal surface presented numerous large, dark red ulcerated areas. Thick shaggy fibrinous exudate covered the serosal surface.

The ovaries and oviducts were uniformly reduced in size. The atrophic uterus contained a round intramural myoma, 3 cm. in diameter, in the fundus.

The adrenal glands appeared normal in size, shape and consistency for a patient of this age.

The brain was not examined because of restrictions on the autopsy.

*Anatomic Diagnosis.*—Acute diffuse gangrenous cystitis with pelvic and early generalized peritonitis; bilateral ascending pyelonephritis; pulmonary congestion and edema; marked atrophy and fibrosis of the stomach; pleural adhesions; chronic perisplenitis; acute splenitis; fatty(?) infiltration of the liver; leiomyoma uteri; marked cachexia.

*Microscopic Examination*—Sections from the midportion of the esophagus showed an irregular thinning of the stratified squamous epithelium as though portions of the more superficial layers had sloughed away. Minor degenerative



Fig. 2.—Stomach as removed at autopsy after excision of a portion on the lesser curvature for histologic study. The cross indicates the approximate region opposite that on the posterior wall from which the tissue shown in figure 3 was taken.

changes were present within the epithelial cells, but inflammatory cells were absent. In the submucosa, small collections of chronic inflammatory cells were scattered about the engorged capillaries and about the mucous glands. Throughout this coat the veins were considerably dilated. Some slight old scarring was present. The muscular coats appeared normal. In portions of the esophagus bordering on the cardia of the stomach, groups of large and small lymphocytes, clasmotocytes and polymorphonuclear leukocytes extended in tongue-like projections from the subepithelial layers well into the underlying muscle coat; here some patchy fibrosis was also present. Sections from the lesser curvature of the stomach immediately below the esophageal junction showed the gastric glands

considerably distorted, atrophied and partly replaced by condensed stromal connective tissue; there was interstitial infiltration by a considerable number of lymphocytes, large mononuclear cells, polymorphonuclear neutrophils and eosinophils. The destruction of the glandular elements increased in the descent along the lesser curvature, and they were completely replaced by stratified squamous epithelium about 1.5 cm. below the esophageal junction. The stroma beneath the new lining epithelium was also infiltrated with chronic inflammatory cells and thrown up into small papillary projections. Sections removed from the greater curvature of the fundus showed the gastric glands and columnar lining cells entirely absent. The mucosa was represented by a continuation of the stratified squamous epithelium covering the esophagus, with varying amounts of chronic inflammatory cells infiltrating the subepithelial tissue. In order to appreciate the extent of these changes fully, a continuous section almost 1 cm. in width was made from the posterior wall of the stomach about 2 cm. above the greater curvature, extending from the cardioesophageal junction through the pyloric ring. This was blocked in paraffin, and several large sections were cut on a special microtome at about 10 microns in thickness and mounted in the routine manner. This yielded full information on the topography and the extent of the changes in various portions of the wall. Stratified squamous lining epithelium was found to extend along this zone from the esophageal junction to within 7 cm. of the pyloric ring. Another smaller section taken from the pyloric region along the greater curvature showed the stratified squamous epithelium reaching to within 3 cm. of the pyloric valve. Throughout all sections striking hypertrophy and fusing of the fibers of the muscularis mucosae were observed, increasing the thickness of this coat in most areas from six to ten or more times its normal diameter. Within this structure were large, irregular bands of hyalinized connective tissue, which also contributed considerably to its increased width. The middle circular muscle coat showed uniform hypertrophy, but this appeared proportionately less marked than that of the muscularis mucosae (fig. 3). Irregularly placed along the deeper layers of the muscularis mucosae were compact aggregates of small lymphocytes surrounding the small blood vessels. Lying parallel to the long axis of the altered mucosal coat were scattered streaklike collections of large and small lymphocytes, polymorphonuclear neutrophils and eosinophils, with occasional plasma and large mononuclear cells. These subepithelial groups of cells penetrated short distances into the superficial portions of the thickened muscularis mucosae. Here and there bits of the squamous mucosal surface were destroyed by an acute suppurative process. Rarely a small round area of necrosis could be found in the muscularis mucosae beneath and touching the cellular zone. These areas were surrounded by a broken thin layer of epithelioid cells. No remarkable changes were present in either the small or the large blood vessels throughout the stomach. The thickness of the stratified squamous epithelial lining surface varied almost continually and frequently was elevated by small buds of supporting connective tissue stroma infiltrated with chronic inflammatory cells. In some zones it maintained a uniform thickness for a distance of several centimeters (fig. 4A), while in an area close by there were alternate high and low zones (fig. 4B). At the pyloric antrum there was again present a continuous glandular gastric mucosa. The glands, however, were observed to be severely infiltrated by large and small lymphocytes, mononuclear cells and occasional polymorphonuclear leukocytes. In a few areas they were partially replaced by small strands of fibrous connective tissue. An occasional group of well preserved parietal cells lined portions of the less severely involved glands.



Fig. 3.—Section taken through an area on the posterior wall, 14 cm. above the pyloric ring; hematoxylin and eosin stain;  $\times 25$ . Note the replacement of normal mucosa by stratified squamous epithelium and the marked thickening of the muscularis mucosae, with presence of inflammatory exudate.



Fig. 4.—*A*, uniformly thick lining of stratified squamous epithelium; hematoxylin and eosin stain;  $\times 160$ . *B*, alternating high and low portions of the altered mucosa and prominent thickening of the muscularis mucosae; hematoxylin and eosin stain;  $\times 160$ .

The great bulk of the cells remaining were, however, of the clear cell type. A few slight nonspecific chronic inflammatory changes were present in the superficial portion of the duodenal mucosa.

In the kidneys, rather broad streaks of polymorphonuclear neutrophils extended through the medullary pyramids from the mucosa of the calices well into the cortex. Large areas of tubular epithelium were destroyed, and clumps of cells filled the lumens. Some bits of necrotic tissue contained clumps of gram-positive cocci. There was moderate sclerosis of the small arterioles but relatively slight glomerular damage.

The urinary bladder showed extensive deep acute ulcers of the mucosa, covered with fibrinopurulent exudate. In many instances the necrotic bases of an ulcer penetrated into the outer layer of the muscle coat. In the exudate morphologically resembling staphylococci were clumps of gram-positive cocci. In a few zones the muscle coat was entirely necrotic and showed little cellular inflammatory reaction.

The liver contained in the midzonal portions of its lobules occasional minute foci of acute necrosis of hepatic cells surrounded by polymorphonuclear neutrophils. No other noteworthy changes were present.

Apart from a small amount of acute inflammatory exudate on the serosa of the duodenum, jejunum and ileum, no remarkable changes were present in the small intestine.

In the spleen there was marked infiltration of the red pulp by large mononuclear cells and occasional polymorphonuclears. A solitary small circumscribed fibrous nodule was present in the pulp.

The heart revealed moderate sclerosis of the coronary arterioles, with patchy stellate areas of fibrosis replacing some muscle bundles.

The thoracic aorta had moderate intimal atherosclerotic changes and slight nuclear "dropping" in the medial coat.

The pancreas showed moderate sclerosis of the arterioles and some increase in the interstitial supporting connective tissue.

The lungs disclosed chronic emphysema with some intra-alveolar edema and chronic passive congestive changes throughout all sections.

The sinusoids of the hilar lymph nodes were packed with large coal-pigment-laden mononuclear cells and clumps of polymorphonuclear neutrophils. One section showed a large dense nodule composed of compact collagenous fibers. This contained some anthracotic pigment. Nonspecific reacting cells surrounded the nodule.

*Microscopic Diagnosis.*—Diffuse gangrenous cystitis with pelvic and generalized peritonitis; bilateral ascending acute suppurative pyelonephritis with abscess formation; chronic diffuse metaplastic gastritis with hypertrophy of the muscle coats; acute focal necrosis of the liver; healed tuberculous(?) hilar lymphadenitis; healed tubercle in the spleen; acute splenitis; slight myocardial fibrosis; slight pulmonary edema; chronic pulmonary emphysema; interstitial fibrosis of the pancreas; leiomyoma uteri; atherosclerosis of the aorta; moderate generalized arteriosclerosis and arteriolosclerosis.

#### COMMENT

The etiologic agent or the combination of factors effecting the remarkable transformation observed in the inner coats of this uniformly small stomach eludes easy identification. The influence of these changes on the general nutritional state and the blood picture is far more readily understood. Small areas of gastric mucosa containing chronically



inflamed glands at the esophageal junction on the lesser curvature and larger zones of similar mucosa clothing the pyloric antrum indicate the acquired nature and the long duration of the principal process. The fact that the deformed appearance of the stomach observed on roentgenologic examination remained unchanged over a period of more than four years supports this assumption. Histologically the lesion exhibited chronicity but lacked any specific character. There was nothing to suggest syphilis in or about the blood vessels or in the exudative or the productive inflammatory reactions. Special staining for both spirochetes and tubercle bacilli yielded negative results. Furthermore, no other organ examined disclosed specific lesions of syphilis or of active tuberculosis. The production of metaplastic epithelial changes in the stomach by immoderate use of alcohol over an extended period was not substantiated by any history of continual alcoholic abuse, nor do the changes resemble those seen in advanced alcoholic gastritis. The possibility that the patient might have ingested some cauterizing substance destroying a large portion of the gastric lining, or that there had been lengthy exposure of the stomach to the effects of roentgen rays, is unsupported by the history. What is more, stomachs of patients who have survived such episodes for a considerable length of time do not show extensive metaplasias of this type. In the absence of evidence of any of these or similar injuries some etiologic factor other than that relating to direct contact must be sought. The long history of a deficient diet and the clinically observed chronic malnutrition and anemia may be of significance in this regard. While the patient had complained occasionally of sore tongue, and atrophic changes had been noted involving the mucosa over the edges and posterior third of the tongue, a history of dysphagia was never elicited, and studies of the blood failed to reveal the type of simple hypochromic anemia usually observed in the Plummer-Vinson syndrome.<sup>1</sup> On the other hand, a long-standing lack of vitamin A-containing food substances conceivably could have altered the growth of the gastric epithelium, initiating early metaplastic changes, and at the same time lowered the resistance of this tissue to infection. Thus a vicious cycle would be instituted, each extension of the inflammatory response to infection reacting on the already damaged gastric mucosal cells and aiding the development of further metaplastic changes, and this in turn impairing nutritional absorption and favoring additional bacterial penetration and inflammation. The increase in thickness of the muscularis mucosae and its scarification by hyalinized connective tissue seem to define the innermost boundary of the preceding inflammation. The increased thickness of the circular muscle coat possibly represents a work hypertrophy of the imperfectly functioning organ. No obstructive mechanism was found responsible for the recurrent attacks of vomiting noted clinically. The loss of the intrinsic antianemic factor with widespread damage to the mucosal coat, together with the absence of the extrinsic substance in the diet accounts for the severe macrocytic anemia. The slight changes in the liver observed microscopically appeared to be of extremely minor importance. Doubtless they were secondary to inflammatory foci in the stomach and had no possible bearing on storage of the "X" hemopoietic

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1. Hoover, W. B.: *New England J. Med.* **213**:394, 1935.

factor in the liver. Should the assumption be correct that chronic lack of dietary vitamin A was instrumental in producing the primary changes in the gastric mucosa, it still fails to explain the absence of other more common clinical manifestations of this deficiency state; nor does it account for the lack of signs and symptoms of other avitaminoses which must have existed to some extent in chronic starvation of this type, unless it is assumed that the supply of these substances was adequate to prevent clinical manifestations. The gradual but definite improvement in the patient's blood picture and general body nutrition on a selected hospital diet indicate that sufficient absorption was maintained through the duodenal and jejunal mucosal coats to overcome the loss of intrinsic factor in the lower portion of the stomach, a fact substantiated by the absence of noteworthy histologic alterations in the mucous membranes of the duodenum and jejunum. .

A final solution to the cause and significance of the changes noted in the stomach of this patient appears to rest on future investigation.

#### SUMMARY

A Negro woman in the fifth decade of life had, during six years, recurrent attacks of vomiting, abdominal pain and progressive general weakness. There was marked cachexia associated with macrocytic anemia which did not respond to liver therapy. Roentgen examinations showed an extremely small and sluggish stomach. Gastroscopecally, the mucosa was pale and rugae were absent. At autopsy the stomach was found to be uniformly reduced in size and its wall regularly thickened with diffuse squamous metaplasia of the mucosa. No apparent cause for the gastric changes was found. The possibility of vitamin A deficiency must be considered.

## FOCAL LIPID GRANULOMATOSIS OF THE LUNG FOLLOWING INSTILLATION OF IODIZED POPPYSEED OIL

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Iodized oil is introduced into the bronchial tree for diagnostic purposes on the assumption that it produces no local reaction, is systemically harmless and is readily disposed of by the body. Clinical experience and some experimental work tend to justify the procedure. However, some contrary evidence exists. Bezançon, Delarue and Valet-Bellot,<sup>1</sup> in a study of lungs from patients dying two days, twelve days and six years after instillation of iodized poppyseed oil demonstrated residual oil but no striking tissue response other than the presence of lipophages. That iodized poppyseed oil can produce a significant reaction in the human lung has been demonstrated, though rarely. Rabinovitch and Lederer<sup>2</sup> "saw such a case following the intratracheal introduction of this substance [iodized poppyseed oil] in an old person who later died of bronchopneumonia. The lungs showed changes similar to those seen in lipoid pneumonia." Unfortunately, these authors gave no further description of the case. Lenk and Haslinger,<sup>3</sup> also without giving any pathologic details, have written of the occurrence of "foreign body pneumonia" following the use of iodized oil. The acute reactions described by Archibald and Brown<sup>4</sup> in the early days of bronchiography with radiopaque substances undoubtedly have no relation to lipoid pneumonia. The only carefully described case with a true tissue reaction ascribed to iodized oil is that of Wright<sup>5</sup>: Twelve months before the patient's death such oil (rapeseed oil plus iodine) was instilled. One of the main lateral branches of the right bronchus was seen to be blocked completely, but the oil passed readily into the lower branches. Roentgenograms taken a week before death showed the oil still present in bronchiectatic cavities in the neighborhood of a relatively radiopaque mass, especially in parts caudal thereto. A tumor, diagnosed as carcinoma, had occluded the main bronchus. Microscopically, fat was found in macrophages. No collagen formation could be demonstrated with the Van Gieson stain. However, a peculiar pleural reaction was found in the neighborhood, which Wright attributed to the oil. Freiman, Engelberg and Merrit,<sup>6</sup> commenting on this case, suggested that the

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From the Department of Laboratories, Beth Israel Hospital.

1. Bezançon, F.; Delarue, J., and Valet-Bellot, M.: *Ann. d'anat. path.* **12**: 229, 1935.

2. Rabinovitch, J., and Lederer, M.: *Arch. Path.* **17**:160, 1934.

3. Lenk, R., and Haslinger, F.: *Klin. Wchnschr.* **4**:1533, 1925.

4. Archibald, E., and Brown, A. L.: *J. A. M. A.* **88**:1310, 1927.

5. Wright, R. D.: *Am. J. Path.* **11**:497, 1935.

6. Freiman, D. G.; Engelberg, H., and Merrit, W. H.: *Arch. Int. Med.* **66**:11, 1940.

picture was that of "the normal walling off of a foreign body that cannot be removed rather than a reaction attributable to the chemical nature of the oil." They suggested that the oil was trapped by the bronchial neoplasm and so the normal removal mechanism was interfered with.

Experiments with animals have vindicated the clinical belief that iodized vegetable oils are relatively innocuous when introduced into the lung. Fried and Whitaker<sup>7</sup> injected iodized oil into the tracheas of cats. They found it well tolerated in amounts up to 1.5 cc. per kilogram. The oil remained in the lungs for long periods, producing only a phagocytic response, without any sclerotic changes. Animals with "snuffles" rid themselves of the oil more rapidly. Pinkerton,<sup>8</sup> using puppies and rabbits, also concluded that iodized vegetable oils were relatively bland. Brown,<sup>9</sup> with cats, also showed that iodized oil stimulated only a phagocytic response, the phagocytes removing the oil along lymphatic channels. Adams,<sup>10</sup> in the discussion of a paper of Cannon's on lipoid pneumonia, mentioned experiments on dogs in which iodized poppyseed oil was introduced intratracheally. This produced local scarring. He commented that in man the oil remained for a considerable time, and that it is not known why sometimes inflammatory reactions occur and sometimes not. Paterson,<sup>11</sup> in an extensive study of the response of lung tissue to oily substances, concluded that poppyseed oil with 40 per cent iodine called forth little reaction. Macrophages took on a finely granular appearance after phagocytosis of the oil, though the amount of phagocytosis was slight. He concluded that in human beings the oil is probably almost completely removed by expectoration. He stated, without giving any of the detailed evidence, that in experimental animals given injections of the aforementioned oil tiny areas of reaction were found after seventy days. Peiper and Klose<sup>12</sup> injected 2 and 3 cc. of 20 per cent iodized sesame oil into 2 guinea pigs. One animal died in twenty-four hours with severe tracheitis and pneumonia; the second was killed after nine days and was found to have severe pneumonia. The histologic details were not described.

I have had the opportunity of studying a lung removed because of a bronchial neoplasm shortly after a diagnostic bronchographic procedure in which iodized poppyseed oil had been instilled into the lung.

A man aged 52 began to have a chronic cough. A relative who was a physician noted the rapid development of clubbing of the fingers. At bronchographic examination 10 cc. of iodized poppyseed oil 40 per cent was used, and an attempt was made to get into the upper lobe of the right lung. The orifice was blocked by a tumor mass, and all of the oil ran into the lower and the middle lobe of the right lung. A few days later, bronchoscopic biopsy revealed squamous cell carcinoma. One month after the initial bronchographic procedure, the right lung was resected. Corresponding to the clinical findings, a neoplasm, diagnosed as

7. Fried, B. M., and Whitaker, L. R.: *Arch. Int. Med.* **40**:726, 1927.

8. Pinkerton, H.: *Arch. Path.* **5**:380, 1928.

9. Brown, A. L.: *Surg., Gynec. & Obst.* **46**:597, 1928.

10. Adams, W. E., in discussion on Cannon, P. R.: *Arch. Path.* **19**:135, 1935.

11. Paterson, J. L. H.: *J. Path. & Bact.* **46**:151, 1938.

12. Peiper, H., and Klose, H.: *Arch. f. klin. Chir.* **134**:303, 1925.

squamous cell carcinoma, partly occluded the right main bronchus. When, immediately after removal, the surgical specimen was examined, numerous small firm nodules, then misinterpreted in the gross as metastases, were found scattered beneath the pleura and in the parenchyma of the middle and lower lobes. The patient had a stormy postoperative course and succumbed to an infection of the wound on the sixth postoperative day. At autopsy, no tumor metastases were found, and the left lung showed no foci similar to those previously seen in the middle and lower lobes of the right lung.

A detailed description of the surgically removed right lung follows.

The lung showed, in addition to the usual three lobes, an azygos lobe. The azygos, the upper and the middle lobe were almost entirely atelectatic (pre-operative pneumothorax). On the other hand, the lower lobe was somewhat emphysematous. The mechanism by which this occurred is to be discussed in a separate paper.<sup>13</sup> Since the granulomatous nodules were found in both the atelectatic middle lobe and the emphysematous lower lobe, these factors per se cannot be related to the genesis of the nodules.

In the right main bronchus a polypoid tumor mass, 2 by 1.5 cm., was found close to the cut edge. It projected into the lumen so as to form a valvelike structure partly obstructing the entrance into the lower lobe and, to a lesser extent, that into the middle lobe. In addition, a small submucosal tumor nodule, about 7 mm. in diameter, further incompletely obstructed the lumen of the bronchus to the middle lobe. Another tumor mass, 1.5 by 1 cm., incompletely separated from the aforementioned polypoid mass, totally occluded the orifice of the bronchus to the upper lobe. Continuous with this was a mass about 4 cm. in diameter, involving all the branch bronchi to the upper lobe and also the branch to the azygos lobe, which arose from the bronchus to the upper lobe. At one point the neoplasm invaded the pulmonary vein.

Scattered beneath the pleura of the middle and lower lobes, as well as throughout their parenchyma, were numerous firm brown or gray-brown nodules. These were ovoid or irregularly spherical, some were confluent, and the largest measured about 1 cm. in greatest diameter. They had sharp boundaries and stood up slightly above the cut surface of the lung. The subpleural ones similarly raised the pleura and were visible to the naked eye. They were perhaps slightly more numerous at the apexes of these lobes, but no special distribution could be recognized.

Microscopically, the tumor was seen to be squamous cell carcinoma, with considerable hornification. No tumor was found anywhere but in the major and adjacent subsidiary masses. The invasion of the pulmonary vein was confirmed.

The nodules in the middle and lower lobes consisted of aggregates of tubercle-like structures. At the centers of many were fat globules, appearing as empty spaces in the paraffin sections, staining orange red with sudan IV and black with osmic acid. Stretched over the periphery of the fat droplet in many instances was a giant cell of the foreign body type. Epithelioid cells and macrophages surrounded these, with a peripheral rim of lymphocytes and fibroblasts. Many of the nodules contained no free fat, but the central core was formed by lipophages and epithelioid cells. About the "tubercle" in many instances a fairly dense collar of collagen was laid down. With silver impregnation, a fine network of fibers surrounded each tiny focus and bound adjacent ones. No argyrophilic fibers were found in

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13. Cohen, A. G.: Personal communication to the author.

the central portions occupied by the lipophages. With Van Gieson and azan carmine stains, fibrous strands were found occasionally crossing an individual unit, and a rare focus appeared entirely replaced by fibrous tissue. Scattered multinucleated giant cells with large fat-containing vacuoles were fairly numerous. In the alveoli adjacent to the involved areas there were clusters of lipophages, and occasional fat-laden macrophages were found in lymphatics at some distance.

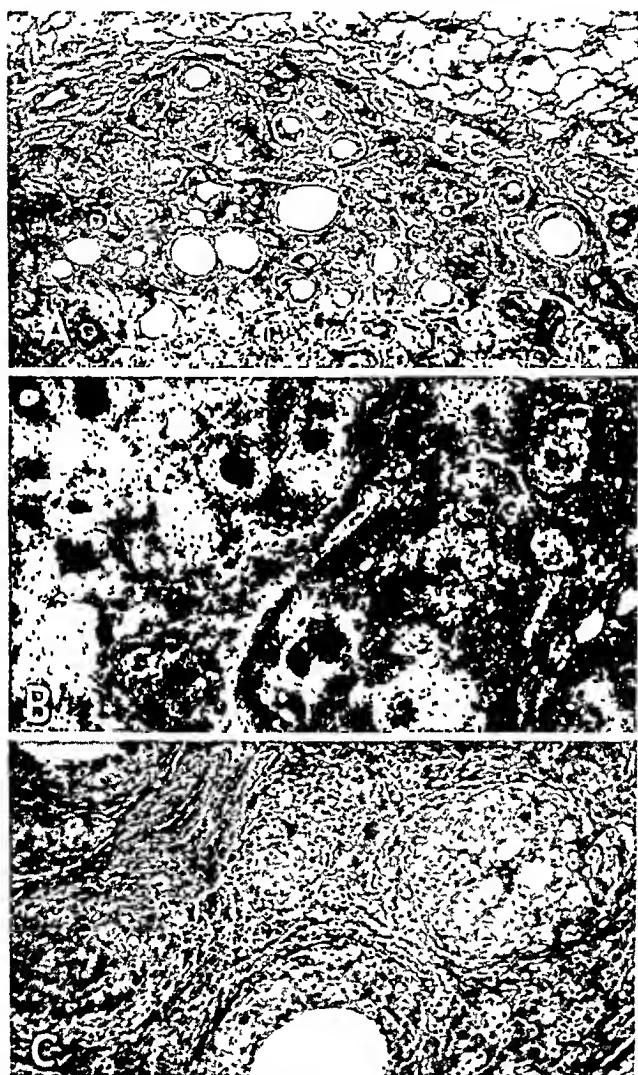


Fig. 1.—*A*, focal granuloma, paraffin section, hematoxylin and eosin stain. *B*, focal granuloma, frozen section, osmic acid stain. *C*, individual tubercle-like structures in granulomatous nodules. Azan carmine stain.

No iron pigment was found in the nodules. Ziehl-Neelsen stain revealed no acid-fast substance such as has been described in cases of cod liver oil pneumonia. However, with this stain, some of the giant cells assumed a slightly pinkish hue, and occasional reddish granules were seen in some of the epithelioid cells. Material preserved in Kaiserling solution, long after the original fixation, was stained with Nile blue sulfate; the free fat stained a deep magenta; the intra-

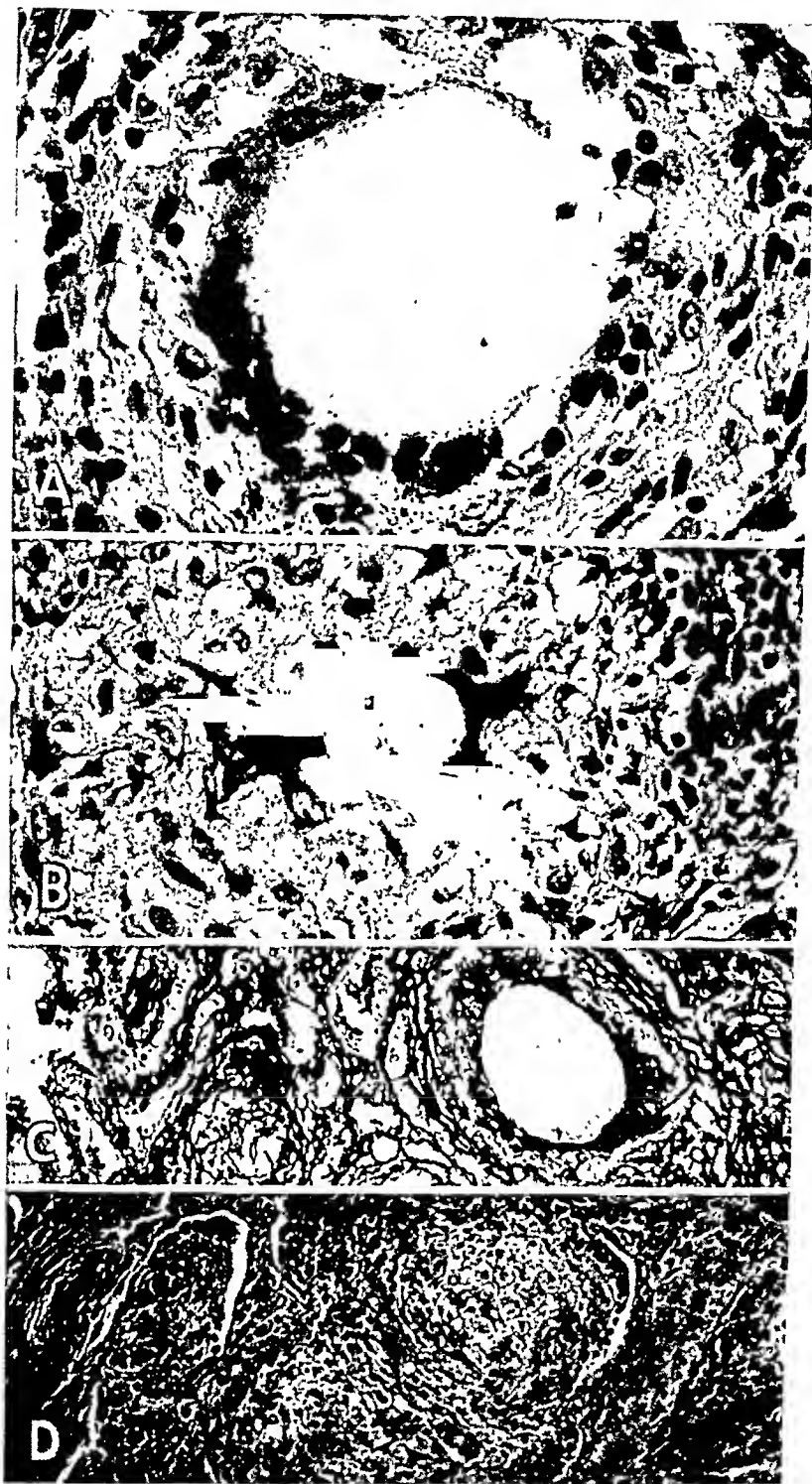


Fig. 2.—*A*, foreign body giant cell stretched over the surface of an oil droplet in the center of a "tubercle." *B*, giant cell containing an oil droplet in its center, surrounded by epithelioid cells and lipophages. Note the similarity to a tubercle. *C*, silver impregnation showing a network of argyrophile fibers about and between "tubercles." *D*, Van Gieson stain showing collagen formation around and between "tubercles."

cellular fat gave a purple-blue color. The interpretation of these reactions after such a long period of fixation seems hazardous. However, the staining would indicate that the free fat was neutral and saturated.

The saturation of the fat (by iodine) is further indicated by the fact that roentgenograms of thin slices showed tiny radiopaque areas that agreed in both distribution and size with the oil droplets seen in microsections. Unfortunately, attempts at extracting the oil and chemically analyzing it were unsuccessful.

The pathologic diagnosis was: squamous cell carcinoma of the main bronchus to the right lung, with occlusion of the bronchus to the upper lobe and partial obstruction of the bronchi to the middle and lower lobes of the lung; atelectasis of the azygos, upper and middle lobes; emphysema of the lower lobe; granulomatosis of the middle and lower lobes (foreign body reaction to iodized poppyseed oil).

#### COMMENT

It seems clear that in this case there occurred a granulomatous reaction to iodized poppyseed oil instilled into the lung. From the survey of the literature and from general clinical experience, this must represent a most unusual result. The only case which is at all similar is that of Wright,<sup>5</sup> and in that no granulomatous proliferation was found in the pulmonary parenchyma. The 2 cases do have in common the trapping of the iodized oil (partly or completely) behind a bronchial tumor. It may well be that this mechanical factor played a role in these 2 cases. However, in the present case the time elapsing between the instillation of the oil and the examination of the lung was only one month. In many cases, iodized oil has been demonstrated in the lung for longer periods without evidence of a similar reaction. I have examined two other pneumonectomy specimens which had previously received iodized poppyseed oil and found no similar lesions. Some other factor, or factors, must be involved. This case throws no immediate light on what these might be. It should draw attention, however, to the fact that instillation of iodized oil into the human lung cannot be carried out without a certain danger of producing a chronic tissue reaction. The possibility that such foci might be misinterpreted as tumor metastases should be borne in mind.

#### SUMMARY

A case with multiple foci of lipid granulomatous reaction in a lung resected for carcinoma of the bronchus one month after diagnostic instillation of iodized poppyseed oil is presented. The lesion was restricted to the lobes into which the oil had been introduced and in which the bronchi were partially obstructed by the tumor.



# Laboratory Methods and Technical Notes

## AN INSTRUMENT FOR REMOVING THE SPINAL CORD THROUGH THE FORAMEN MAGNUM

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The need for routine examination of the spinal cord in complete autopsies led to the development of an instrument which facilitates removing the cord from within the cranial cavity without the necessity of removing the spinal processes, laminae and dura. The instrument now in use is a modification of one described by Brines, Webster and Chesney<sup>1</sup> and was evolved after numerous types of blades had been tried.

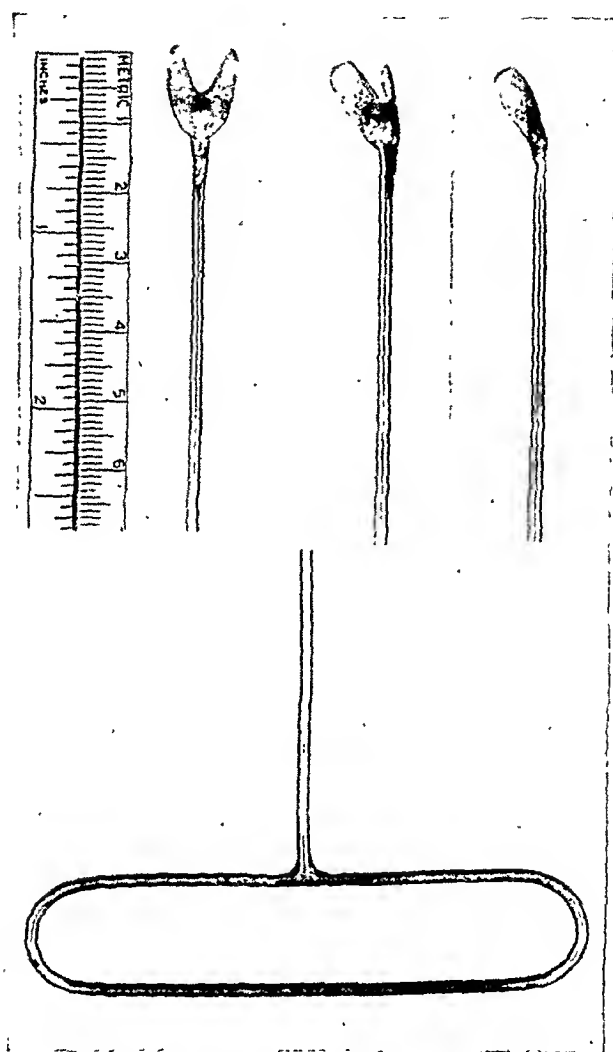
This instrument consists of a blade mounted on a spring steel shaft measuring 50 cm. in length and 0.2 cm. in diameter. At the opposite end the shaft is bent into a loop, which forms the handle. The blade consists of a V-shaped piece of German silver measuring 1.3 cm. in length and 1.5 cm. in width. The apex is united to the shaft by a rivet, followed by soldering. Union by either method alone is not satisfactory. The two arms of the V are bent forward so that they make an angle of about 45 degrees with each other and form between them a smaller V-shaped recess which has sharpened edges. The outer ends and edges are rounded and dull, so that this portion of the instrument will not impinge on the bony walls of the vertebral canal or incise the cord itself. The arms of the V-shaped blade make an angle of about 25 degrees with the axis of the shaft, though the apex formed by the cutting edges is on a line with the shaft.

The cord is removed in the following manner: After removal of the brain the anterior cervical and dorsal thoracic curves are straightened by placing a block beneath the thoracic portion of the spinal column and by elevating the occipital portion of the skull. The upper roots on one side of the divided spinal cord are grasped with a Mayo clamp, and slight tension is placed on the cord. The instrument is then pushed along one side of the cord within the subarachnoid space with its concave surface facing the convex lateral surface of the spinal cord. As the instrument passes down the vertebral canal, the motor and sensory roots and the dentate ligament are gathered into the V-shaped recess and are severed by its sharpened inner edges. When the blade has severed the roots down to the lumbar region, it is removed and the roots on the opposite side are divided in a similar manner. The lower end of the cord, or cauda equina, is transected after exposure of the vertebral canal by transecting one of the intervertebral disks at the appropriate level. The cord is then gently removed by pulling it upward through the foramen magnum.

With this procedure the spinal cord may be removed satisfactorily for routine examination with little or no gross or histologic evidence of trauma. The instrument has been used successfully in cadavers of all ages from 6 to 7 months upward. Smaller but similarly constructed blades might easily be substituted for use in smaller infants or animals. The instrument works poorly, however, in cases in which there is marked thoracic kyphosis, scoliosis or hypertrophic arthritis. In these cases and when an expanding lesion of the cord which would obliterate the spinal canal is suspected, i. e., a neoplasm, it is preferable to expose the cord in the usual manner by removing the posterior portion of the spinal column.

From the Division of Pathology, University of California Medical School.

1. Brines, O. A.; Webster, J. E., and Chesney, A.: Arch. Path. 22:390, 1936.



Above: anterior, oblique and lateral views of the blade. Below: loop handle of the shaft.

# General Reviews

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## MORPHOLOGIC, PHYSIOLOGIC, CHEMICAL AND BIOLOGIC DISTINCTION OF MEGALOBLASTS

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For many years European hematologists have utilized the recognition of genuine megaloblasts as an aid in the diagnosis of severe anemic conditions. This is due primarily to the excellent publications by Pappenheim, Naegeli and Ferrata. Secondly, it is because most of the European hematologists have used blood and marrow from patients with pernicious anemia as their source of prototype megaloblasts. Some hematologists in the United States have failed to recognize the genuine megaloblast as a pathologic type of erythropoietic cell and consequently have not appreciated the significance of finding these cells in either sternal marrow or the peripheral blood. This misconception of erythropoiesis is due to the widespread acceptance of the theory of erythropoiesis advanced by Doan, Cunningham and Sabin,<sup>1</sup> which has been supported in part by Isaacs<sup>2</sup> and Osgood.<sup>3</sup> This theory is inadequate and unsuited for the proper interpretation and explanation of erythropoiesis under normal and pathologic conditions in man for several reasons. First, it is based on Sabin's<sup>4</sup> studies of blood cells in the blastoderm of the chick embryo, which are not morphologically identical with genuine megaloblasts and are not present in normal chick marrow.<sup>5</sup> Secondly, Doan.

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From the Department of Anatomy of the University of Buffalo School of Medicine.

1. Doan, C. A.; Cunningham, R. S., and Sabin, F. R.: *Contrib. Embryol.* **16**: 163, 1925.

2. Isaacs, R.: The Erythrocytes, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 1, p. 5. In a recent personal communication Isaacs indicated that he has changed his views regarding megaloblast. He now believes that when the megaloblast does not mature in the absence of hepatic principle, it undergoes marked changes in nuclear and cytoplasmic area as it lingers in the marrow. The end result is that a larger erythrocyte is produced. He also believes there are certain analogies to tumor cell formation in the body.

3. Osgood, E. E., and Ashworth, C. M.: *Atlas of Hematology*, San Francisco, J. W. Stacy, Inc., 1937.

4. Sabin, F. R.: *Bull. Johns Hopkins Hosp.* **32**:314, 1921.

5. Jones, O. P.: Unpublished data.

Cunningham and Sabin indiscriminately redefined the term "megalo-blast" to include primitive erythroblasts of the embryonic yolk sac, pronormoblasts (proerythroblasts) of the fetal liver and normal marrow and promegaloblasts of the marrow of patients with pernicious anemia.<sup>6</sup> Maximow<sup>7a</sup> attempted to justify his own misuse of the term "megalo-blast"<sup>7b</sup> as well as that of Doan, Cunningham and Sabin by claiming no reference was made to the cell's being related to the pathologic erythroblast of pernicious anemia or to "Ehrlich's megaloblast." This does not hold for Sabin and Miller,<sup>8</sup> since they are of the opinion that the youngest red cells in normal rabbit marrow are of the same type as the most immature erythroid forms seen in the blood in pernicious anemia. Thirdly, in view of the dominant position which the megaloblast has in the theory of erythropoiesis advanced by Doan, Cunningham and Sabin, it is disconcerting to find no record that they ever studied tissue known to possess genuine megaloblasts while they were formulating their theory from 1921 to 1925.<sup>5</sup> And finally, the so-called confirmation of the Doan, Cunningham and Sabin theory by Peabody,<sup>9</sup> Muller,<sup>10</sup> Sabin and co-workers<sup>11</sup> and Sabin and Miller<sup>8</sup> was based on a study of marrow in which genuine megaloblasts are known to be lacking.<sup>5</sup> It should be realized that this criticism is leveled toward only one part of their theory and does not take into consideration the arguments regarding the retention of embryonic potencies by endothelial cells in the adult<sup>12</sup> nor the presence or the absence of intersinusoidal capillaries in normal marrow.<sup>13</sup> Jordan and Johnson<sup>14</sup> have made certain remarks concerning the confirmation of the Doan, Cunningham and Sabin concept of erythropoiesis which bear repetition, viz.: "It seems desirable that a result of such magnitude, considering its wide application especially in

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6. Jones, O. P.: (a) *Anat. Rec. (supp. 3)* **70**:42, 1938; (b) *Proc. Soc. Exper. Biol. & Med.* **38**:222, 1938.

7. Maximow, A.: (a) *Bindegewebe und blutbildende Gewebe*, in von Mollendorf, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1927, pt. 1, p. 232; (b) *Folia haemat.* **4**:612, 1907; *Arch. f. mikr. Anat.* **73**:444, 1909.

8. Sabin, F. R., and Miller, F. R.: *Normal Bone Marrow*, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 1791.

9. Peabody, F. W.: *Am. J. Path.* **2**:487, 1926.

10. Muller, G. L.: *J. Exper. Med.* **43**:533, 1926; **45**:399, 1927; *Am. J. Physiol.* **82**:269, 1927.

11. Sabin, F. R.; Miller, F. R.; Smithburn, K. C.; Thomas, R. M., and Hummel, L. E.: *J. Exper. Med.* **64**:97, 1936.

12. Bloom, W.: *The Embryogenesis of Mammalian Blood*, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 863.

13. Ringoen, A. R.: *Anat. Rec. (supp. 4)* **58**:84, 1934; (supp. 4) **61**:42, 1935. McDonald, J. G.: *Am. J. Anat.* **65**:291, 1939.

14. Jordan, H. E., and Johnson, E. P.: *Am. J. Anat.* **56**:71, 1935.

pathology, should be further tested by additional investigations. There are few similar instances where a concept of such wide and important bearings has been so completely accepted with so little effort at verification."

#### MORPHOLOGIC DISTINCTION OF MEGALOBLASTS

Evidence has been accumulating steadily in support of the contention that megaloblasts are found almost entirely, if not exclusively, in patients with macrocytic anemia of the type due to a deficiency of liver principle. Limarzi<sup>15</sup> found this to be so in a series of 2,215 or more sternal marrows, and Rohr,<sup>16</sup> in a series of more than 1,800. Hence, to the disciplined eye the presence of a megaloblastic marrow not only separates this type of anemia from other types of macrocytic anemia but means that in an uncomplicated case the patient can be treated efficaciously with potent liver extract or desiccated porcine stomach.<sup>17</sup>

It is not the purpose of this paper to discuss at length the terminology, the structure and the origin of megaloblasts, their relationship to primitive erythroblasts of the yolk sac or their fate after liver therapy, for these subjects are treated elsewhere.<sup>18</sup> However, it should be borne in mind that the genuine megaloblast is the predominant type of red cell developed in the marrow of patients with pernicious anemia during severe relapses. The youngest megaloblasts are usually derived from the myeloblasts and rarely directly from the reticuloendothelium.<sup>18a</sup> The end products of the megaloblastic series are reticulated and mature megalocytes, which are responsible for the increases in color index, corpuscular diameter and mean corpuscular volume in the peripheral blood of patients with pernicious anemia.<sup>6a</sup>

The consideration of the megaloblastic series as a pathologic type of erythropoiesis is based on the following morphologic evidence. The

15. Limarzi, L. R.: Personal communication to the author.

16. Rohr, K.: (a) *Knochenmarksmorphologie des menschlichen Sternalpunktats*, Berlin, Urban & Schwarzenberg, 1937; (b) *Das menschliche Knochenmark*, Leipzig, Georg Thieme, 1940.

17. (a) Koller, F.: *Deutsches Arch. f. klin. Med.* **184**:568, 1939. (b) Wilson, T. E.: *M. J. Australia* **29**:513, 1942. (c) Watson, C. J.: *Illinois M. J.* **82**:195, 1942.

18. (a) Jones, O. P.: *Cytology of Pathologic Marrow Cells*, with Special Reference to Bone Marrow Biopsies, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 2045. (b) Rohr.<sup>16</sup> (c) Fieschi, A.: *Semeiologia del midollo osseo: Studio di morfologia clinica*, in Ferrata, A.: *Biblioteca "Haematologica"*, Pavia, Tipografia gia cooperativa, 1938; *Ergebn. d. inn. Med. u. Kinderh.* **59**:382, 1940. (d) Schulten, H.: *Die Sternalpunktion als diagnostische Methode*, Leipzig, Georg Thieme, 1937. (e) Maximow.<sup>7a</sup> (f) Naegeli, O.: *Wien. klin. Wchnschr.* **48**:225, 1935. (g) Koller.<sup>17a</sup> (h) Bock, H. E., and Malamos, B.: *Folia haemat.* **62**:408, 1939. (i) Schartum-Hansen, H.: *ibid.* **58**:145, 1937. (j) Lambin, P., and de Weerd, W.: *Rev. belge sc. méd.* **10**:282, 1938.

nuclear structure of the promegaloblast is quite different from that of any cell in normal marrow and especially that of the pronormoblast (proerythroblast).<sup>19</sup> It is composed of a chromatin network which is very finely stippled or divided into small thickened knots and masses. These are uniformly distributed throughout the nucleus and are separated by an equal or even greater amount of slightly acidophilic parachromatin. A tendency for the chromatin to clump is found only in the vicinity of the nucleoli, which are faint and not sharply outlined.<sup>20</sup> Even though the vast majority of promegaloblasts can be traced back to an origin from myeloblasts (lymphoidocytes),<sup>21</sup> they do have a nucleus which bears some resemblance to that found in histiocytes or reticulum of the hemopoietic tissue. This peculiar reconstruction of the nuclear pattern after cells have passed beyond the myeloblast (lymphoidocyte) stage may be the reason some authors have believed all megaloblasts in all cases are derived directly from the reticuloendothelium.<sup>21f</sup> In the later stages of megaloblastic development, when the cytoplasm is polychromatophilic or acidophilic, the chromatin has a tendency to fuse into larger discrete clumps, which may be connected to one another by fine threads of chromatin. These irregular chromatin masses are surrounded by an abundant acidophilic parachromatin, which gives the nucleus a dappled or speckled appearance. The general pattern in megaloblastic nuclei persists even though the cytoplasm is completely acidophilic. Remnants of this characteristic nuclear pattern remain discernible until shortly before pyknosis.<sup>22</sup> This type of nuclear pattern is never found in cells of the normoblastic series. The diameter of young megaloblasts may reach 40 microns, which is much greater than the usual 15 to 25 microns of pronormoblasts in normal marrow.<sup>23</sup> The nucleocytoplasmic ratio is disturbed by a marked increase in the amount of cytoplasm. The latter has a peculiar deep blue basophilia, which is not as homogeneous as the basophilia of pronormoblasts.<sup>18a</sup>

19. (a) Jones,<sup>18a</sup> plates I, III and IV. (b) Wintrobe, M. M.: *Clinical Hematology*, Philadelphia, Lea & Febiger, 1942, plate 2, figs. 10 and 12. (c) Fallon, M.: *Classification of the Anemias and Anemias in Infants and Children*, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 2175, plate 1. (d) Maximow, A., and Bloom, W.: *A Textbook of Histology*, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 90, fig. 71.

20. Jones.<sup>18a</sup> Rohr.<sup>16</sup> Jones.<sup>5</sup>

21. (a) Jones, O. P.: *Anat. Rec. (supp. 4)* **58**:23, 1934; (b) (supp. 4) **61**:28, 1935; (c) *The Origin and Structure of Erythroblasts with Special Reference to the Megaloblast*, Thesis, University of Minnesota, 1935; (d) *Proc. Soc. Exper. Biol. & Med.* **34**:694, 1936. (e) Jones.<sup>18a</sup> (f) Tischendorf, W.: *Deutsches Arch. f. klin. Med.* **187**:556, 1941.

22. Jones.<sup>18a</sup> Jones.<sup>5</sup> Rohr.<sup>16</sup> Klima, R.: *Sternalpunktion und Knochenmarks-bild bei Blutkrankheiten*, Berlin, Urban & Schwarzenberg, 1938.

23. Rohr.<sup>16</sup> Jones.<sup>5</sup>

Neither multipolar mitoses nor multinucleated giant megaloblasts are uncommon.<sup>24</sup> The chromosomes in promegaloblasts and basophilic megaloblasts tend to be finer and more slender than those of normoblasts.<sup>25</sup> However, even greater alterations of chromosomes and nuclear activities are found in the later stages of megaloblastic development, when the cells are more fully hemoglobinized. There is a tendency for degenerative mitoses to occur in acidophilic megaloblasts which are approaching pyknosis. The chromosomes are much shorter and stumper than normal. In some instances pyknosis and fusion of chromosomes bring about an apparent modification of the number. Fieschi<sup>18c</sup> suggested that perhaps cells which are undergoing rapid proliferation pass beyond the stage in which pyknosis and denucleation normally take place, thereby accounting for the abnormal chromosomes. Or the abnormality may be related to the physicochemical state of the protoplasm, which is impossible to determine accurately. Fieschi<sup>26</sup> has also described a lagging behind of some chromosomes during mitosis, which gives rise to aberrant chromatin masses. The end result of such a mechanism has been seen in a case in which, following the reconstruction phase, there were two extranuclear chromosomes in the cytoplasm adjacent to an intact nuclear membrane. These extranuclear chromosomes were found in a basophilic megaloblast, and the phenomenon has not been observed in any other type of blood cell so far.<sup>5</sup>

Although hemoglobiniferous cells are for the most part devoid of any cytoplasmic granulation when routine hematologic stains are employed, there have been exhibited basophilic megaloblasts with atypical azurophilic granulation in a few cases of pernicious anemia.<sup>27</sup> A somewhat different type of granulation has been found in acidophilic megaloblasts after liver therapy.<sup>6b</sup> Recently Sergerdahl<sup>28</sup> reported that this peculiar vaculogranular degeneration of megaloblasts is seen quite regularly in cases of pernicious anemia of pregnancy. As to whether all of these cytoplasmic structures are the same is a subject for further investigation. The late stages of megaloblastic development usually show marked karyorrhexis or karyolysis or both. Karyolysis may lead to the formation of Cabot's ring bodies, which have been found even in hemo-

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24. (a) Rohr.<sup>16</sup> (b) Fieschi, A.: *Haematologica* **17**:125, 1936. (c) Fieschi.<sup>18c</sup> (d) Limarzi, L. R.; Levinson, S. A., and Jones, R. M.: *J. A. M. A.* **118**:1004, 1942. (e) Limarzi, L. R.; Jones, R. M., and Levinson, S. A.: *Anat. Rec. (supp. 3)* **79**:43, 1941.

25. Rohr.<sup>16</sup> Fieschi.<sup>18c</sup> Schwarz (*Am. J. Cancer* **33**:120, 1938) was able to show that the diploid number of 48 chromosomes in man also holds for megaloblasts.

26. Fieschi.<sup>18c</sup> Fieschi.<sup>24b</sup>

27. (a) Segerdahl, E.: *Ueber Sternalpunktionen*, Uppsala, Appelbergs Boktryckeriaktiebolag, 1935. (b) Jones, O. P.: *Folia haemat.* **55**:195, 1936.

28. Sergerdahl, E.: *Acta med. Scandinav.* **108**:483, 1941.

globiniferous cells with an intact nucleus.<sup>29</sup> However, these views have been discredited in the recent work by Schleicher,<sup>29a</sup> who asserted that Cabot ring bodies are not nuclear remnants and are not identical with the nuclear membrane but are laboratory creations—the expression of cellular degeneration induced by hemolytic agents. Cabot ring bodies represent an expression of aggregated and denatured colloid protein.

The megaloblasts of pernicious anemia and the primitive erythroblasts of mammalian embryos have been differentiated from each other on the basis that the latter fail to enucleate rapidly and seldom undergo karyorrhexis.<sup>30</sup> Livadas<sup>31</sup> described the embryonic cells as larger, more hyperchromic and having more luster and gloss. The pathologic megaloblasts also differ from primitive erythroblasts in that they are formed extravascularly in the marrow, the liver and the spleen along with the abnormal neutrophils, whereas the primitive erythroblasts are formed intravascularly only in the embryonic yolk sac under physiologic conditions.<sup>32</sup> Moreover, recent experiments indicate that primitive erythroblasts—quite unlike megaloblasts—will respond to massive doses of iron alone.<sup>32a</sup>

The end products of the megaloblastic series are non-nucleated and larger and contain more hemoglobin per corpuscle than normal erythrocytes. Jaffé<sup>33</sup> described the megalocyte as “a real functional giant which is doomed to quick destruction, since it is an abnormal cell.” Megalocytes also differ from normal erythrocytes in that they are more oval.<sup>34</sup> Most megalocytes pass through a stage in which they have reticulation; some, however, do not.<sup>6</sup>

#### PHYSIOLOGIC AND CHEMICAL DISTINCTION OF MEGALOBLASTS

Morphologically the marrow in pernicious anemia is characterized chiefly by very active proliferation of hemoglobiniferous cells foreign to normal marrow, which are in all phases of maturation. This is contrary

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29. Isaacs.<sup>2</sup> Tsamboulas, N., and Malikiosis, X.: *Deutsches Arch. f. klin. Med.* **184**:183, 1939.

29a. Schleicher, E. M.: *J. Lab. & Clin. Med.* **27**:983, 1942.

30. Kirschbaum, A.: *Blood Cell Formation in Mammalian Embryos from the First Appearance of Vascular Cellular Elements Through the Period of Hepatic Hemopoiesis*, Thesis, University of Minnesota, 1936; *Proc. Soc. Exper. Biol. & Med.* **35**:542, 1937.

31. Livadas, K.: *Folia haemat.* **49**:365, 1933.

32. Maximow.<sup>7a</sup> Naegeli.<sup>18f</sup> Lividas.<sup>31</sup> Rohr.<sup>16</sup> Jones.<sup>5</sup>

32a. Jones, O. P.: *Anat. Rec.* **85**:321, 1943. These investigations were aided by a grant from the Committee on Scientific Research of the American Medical Association.

33. Jaffé, R. H.: *J. A. M. A.* **107**:124, 1936.

34. Schulten.<sup>18d</sup> Scharf-Hansen.<sup>18i</sup> Rohr.<sup>16</sup> Koller.<sup>17a</sup> Isaacs.<sup>2</sup> Schulten, H.: *Folia haemat.* **57**:189, 1937.



to Peabody's view that there is a proliferation and maturation arrest of the young hemoglobiniferous cells normally present in marrow.<sup>35</sup> Peabody's misconception regarding the erythropoiesis occurring in pernicious anemia during relapse is partly due to the fact that he had little or no faith in the use of dry smears or imprints for studies of marrow. When Peabody<sup>35d</sup> used sections alone, he sacrificed accurate identification of cells for knowledge of the general structure of marrow.<sup>3</sup> The value of dry smears for demonstrating nuclear structure has been reemphasized recently by Sundberg and Downey<sup>36</sup> and even by Cunningham's co-worker, Schwind.<sup>37</sup> The fact that Peabody did not accurately identify the cells of normal marrow or properly interpret the cells that develop in the marrow during relapse in pernicious anemia does not detract from his brilliant work of showing that marrow of the latter type returns to normal after the administration of liver.

Not only is there morphologic evidence to support the concept of the megaloblastic series representing a pathologic type of erythropoiesis but there is some which indicates that these cells are both chemically and physiologically abnormal. Studies of the metabolism of pigment (specifically the rate of excretion of coproporphyrin I) have clearly demonstrated that in pernicious anemia the marrow is abnormally active during relapse and less so during remission.<sup>38</sup> This is further evidence against Peabody's concept of an arrest of maturation during relapse in pernicious anemia. The megaloblastic cells which are responsible for this activity consume oxygen at a rate which is below the lower limit of the normal range for sternal marrow. In contrast to this, normoblastic (erythroblastic) marrow from patients with simple hypochromic anemia has a rate two or three times the normal.<sup>39</sup> Schretzenmayer and Bröcheler<sup>39</sup> maintained that this supports Naegeli's contention that there is a definite difference between megaloblasts and normoblasts (erythroblasts). It is difficult to reconcile the difference in consumption of oxygen if substantia granulofilamentosa (reticulation) plays a role in

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35. (a) Jones.<sup>18a</sup> (b) Jones, O. P.: *Arch. Int. Med.* **60**:1002, 1937. (c) Fieschi.<sup>18c</sup> (d) Peabody, F. W.: *Am. J. Path.* **3**:179, 1927.

36. Sundberg, D., and Downey, H.: *Am. J. Anat.* **70**:455, 1942.

37. Schwind, J. L.: *Anat. Rec. (supp. 2)* **79**:55, 1941. In a personal communication Schwind (1942) expressed the opinion that the misunderstanding of the nature of megaloblasts was due in part to the excessive enthusiasm about the possibilities of the supravital method and the use of this method for purposes to which it is not well adapted.

38. (a) Watson, C. J.: *J. Clin. Investigation* **16**:383, 1937; (b) The Pyrrol Pigments with Particular Reference to Normal and Pathologic Hemoglobin Metabolism, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 4, p. 2447. (c) Dobriner, K., and Rhoads, C. P.: *J. Clin. Investigation* **17**:95, 1938.

39. Schretzenmayer, A., and Bröcheler, H.: *Klin. Wchnschr.* **15**:998, 1936.

the respiration of cells, since both cell types possess an abundance of it in their early developmental stages. Bock and Malamos<sup>18h</sup> studied 17 specimens of sternal marrow taken from a patient with pernicious anemia at intervals throughout a three and a half month period. Their studies of the marrow's consumption of oxygen revealed a high value prior to treatment and a marked decrease four days later. On the sixth day following the start of therapy, when the reticulocytes were at their peak, the consumption of oxygen increased again. Although Bock and Malamos found it difficult to explain this secondary increase when the cells were becoming more mature, it can very likely be attributed to the high percentage of reticulocytes rather than to some unknown respiratory function in hemoglobin-laden erythroblasts, as they described it. The obvious discrepancy between the investigations of these two groups of workers indicates a necessity for further work along these lines. Damblé<sup>40</sup> reported that the oxygen consumption of the megalocytes of pernicious anemia as well as that of the erythrocytes of secondary hypochromic anemia with nonreactive marrow is less than normal, but that the oxygen consumption of the erythrocytes of secondary hypochromic anemia with a reactive marrow is almost twice normal. He expressed the belief that these differences are dependent on the erythropoietic function of the marrow. Henderson,<sup>41</sup> Richards and Strauss<sup>42</sup> and Isac, Matthes and Yamanaka<sup>43</sup> have all shown that the hemoglobin of megalocytes in the blood of patients with pernicious anemia has a smaller affinity for oxygen than the hemoglobin of normal human blood. Deutsch<sup>44</sup> found that the increase of respiration produced in the blood of pernicious anemia by stimulation of the respiratory supplement (Ringer solution extract of fresh rat liver) is far less than that of normal blood. He claimed this may be accounted for by an alteration of the structure of the cell surface on which the oxidation takes place.

The permeability of megalocytes to dextrose, malonamide and thiourea is increased, whereas glycerin permeates more slowly.<sup>45</sup> Bang and Ørskov<sup>45</sup> explained this by assuming that the structure or the function of the cell membrane has a peculiar quality. The megalocytes of pernicious anemia have abnormalities in chemical composition which are indicative of a deficient corpuscular structure.<sup>46</sup> These abnormalities

40. Damblé, K.: *Ztschr. f. d. ges. exper. Med.* **86**:595, 1933.

41. Henderson, L. J.: *Blood: A Study in General Physiology*, New Haven, Yale University Press, 1928.

42. Richards, D. W., and Strauss, M. L.: *J. Clin. Investigation* **4**:105, 1927.

43. Isac, C.; Matthes, K., and Yamanaka, T.: *Arch. f. exper. Path. u. Pharmacol.* **189**:615, 1938.

44. Deutsch, W.: *Biochem. J.* **28**:2002, 1934.

45. Bang, O., and Ørskov, S. L.: *J. Clin. Investigation* **16**:279, 1937.

46. Williams, H. H.; Erickson, B. N.; Bernstein, S.; Hummel, F. C., and Macy, I. G.: *J. Biol. Chem.* **118**:599, 1937.

are excessive amounts of cholesterol esters and a deficiency of phospholipid and free cholesterol. The cation and anion content, chiefly potassium and hemoglobin, respectively, are both increased. Williams and co-workers<sup>46</sup> claimed that "if one accepts the current view with regard to physiological activity, the lipid and mineral composition of the abnormal erythrocytes in pernicious anemia during relapse indicates that the cells are not only in a state of lowered function or activity, but degenerating and retrogressing." After treatment for pernicious anemia there is an elevation in total phospholipids, in cephalin and in sphingomyelin to values above normal, with the amount of lecithin definitely decreased.<sup>47</sup> Megalocytes have been found to have a high magnesium content in the majority of cases of pernicious anemia examined during relapse.<sup>48</sup> This is a property of newly formed cells which may also be found in anemias due to other causes. However, Bang and Ørskov<sup>48</sup> expressed the belief that this lends support to their view that the increased destruction in pernicious anemia is brought about through abnormal fragility and short lifetime of red cells in the marrow and the blood stream. Whipple<sup>49</sup> has maintained for many years that there is a scarcity of building material for the stroma or an abnormality of the cells within the marrow that control the output of framework for the red blood cells.

Seggel<sup>50</sup> recently showed that the red fluorescent erythrocytes are less numerous than normal and frequently lacking in the blood of patients with pernicious anemia during relapse. This phenomenon is accompanied by a low value for the protoporphyrin content of erythrocytes. In general, the amount of red fluorescence in aspirated marrow is no greater than that found in the circulating blood. However, in rare isolated instances rose fluorescence was found in the cytoplasm of leukocytes and orange fluorescence in the nuclei of very young hemoglobiniferous cells. This is a distinct contrast to the absence of fluorescence in nucleated red cells and the grayish white to grayish blue fluorescence of leukocytes in normal bone marrow.

#### BIOLOGIC DISTINCTION OF MEGALOBLASTS

There are at least two substances which will cause megaloblasts to exhibit entirely different biologic reactions. The first is anti-pernicious-anemia principle and the second is arsenic. The exact nature of what happens to the pathologic marrow of patients with pernicious anemia during the first twenty-four to forty-eight hours after the administration

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47. Williams, H. H.; Erickson, B. N.; Bernstein, S. S., and Macy, I. G.: Proc. Soc. Exper. Biol. & Med. **45**:151, 1940.

48. Bang, O., and Ørskov, S. L.: J. Clin. Investigation **18**:497, 1939.

49. Whipple, G. H.: Arch. Int. Med. **29**:711, 1922; J. A. M. A. **104**:791, 1935.

50. Seggel, K. A.: Ergebn. d. inn. Med. u. Kinderh. **58**:582, 1940.

of specific antianemic therapy is still a moot question. It has been demonstrated conclusively by Weiner and Kaznelson,<sup>51</sup> Peabody,<sup>35d</sup> Nordenson,<sup>52</sup> Koller,<sup>17a</sup> Rohr,<sup>16</sup> Scott<sup>53</sup> and others that the megaloblastic marrow of relapse is eventually transformed into a normal-appearing marrow. The unsettled question is: By what mechanism does this dramatic conversion or transformation take place? Some maintain that megaloblasts complete their maturation as megalocytes and are not transformed into cells of the definitive or normoblastic series.<sup>54</sup> The latter reappear and return to normal function by heteroplastic development from the reticulum or the myeloblasts or both and by homoplastic development from the quiescent remnants of the normoblastic series present in the marrow during relapse.<sup>55</sup> Some support for the view that megaloblasts are unable to differentiate into cells of the normoblastic series is found in the recent experiments on the placental transfer of anti-pernicious-anemia principle<sup>56</sup>; one must bear in mind, however, that megaloblasts appear under pathologic conditions and that primitive erythroblasts appear under the physiologic conditions of the embryo.<sup>57</sup> Others have expressed the belief that megaloblasts, which previously lacked sufficient anti-pernicious-anemia principle to mature, are completely transformed into cells of the normal or definitive series after the institution of therapy.<sup>58</sup> Dameshek and Valentine<sup>59</sup> suggested there may be reestablishment of erythropoiesis along normal lines together with direct modification of megaloblasts to normoblasts. Wilson<sup>17b</sup> found no reason why, following liver therapy, the pathologic megaloblasts cannot be transformed into normoblasts or cells resembling normoblasts, which

51. Weiner, W., and Kaznelson, P.: *Folia haemat.* **32**:233, 1926.

52. Nordenson, N. G.: *Studies on Bone Marrow from Sternal Puncture*, Stockholm, Börtzells, Esselte, 1935.

53. Scott, R. B.: *Quart. J. Med.* **8**:127, 1939.

54. (a) Fieschi.<sup>18c</sup> (b) Mustafa, K.: *Ztschr. f. klin. Med.* **136**:416, 1939. (c) Naegeli.<sup>18f</sup> (d) Rohr.<sup>16</sup> (e) Segerdahl.<sup>27a</sup> (f) Storti, E.: *Haematologica* **18**:1, 1937; *Gazz. d. osp.* **59**:807, 1938. (g) Tischendorf.<sup>21f</sup> (h) Jones.<sup>18a</sup> (i) Jones.<sup>6b</sup> (j) Jones, O. P.: *Arch. Int. Med.* **68**:476, 1941. (k) Israëls, M. C. G.: *Lancet* **2**:207, 1941.

55. Jones.<sup>6b</sup> Jones.<sup>54j</sup> Fieschi.<sup>18c</sup> Tischendorf.<sup>21f</sup> Israëls.<sup>54k</sup>

56. Jones.<sup>5</sup> Jones.<sup>54j</sup> Jones, O. P.: *Anat. Rec. (supp. 3)* **73**:29, 1939; (supp. 2) **76**:34, 1940; (supp. 3) **79**:35, 1941; (supp. 3) **82**:77, 1942.

57. Recently these investigations have been aided by a grant from the Committee on Scientific Research of the American Medical Association.

58. (a) Doan, C. A.: *Medicine* **10**:323, 1931; *J. Lab. & Clin. Med.* **17**:887, 1932; *Bull. New York Acad. Med.* **15**:668, 1939. (b) Henning, N.: *Deutsche med. Wchnschr.* **39**:1543, 1935. (c) Houghton, B. C., and Doan, C. A.: *Am. J. Clin. Path.* **11**:144, 1941. (d) Isaacs.<sup>2</sup> (e) Osgood.<sup>3</sup> (f) Lambin and de Weerd.<sup>18j</sup> (g) Peabody.<sup>35d</sup> (h) Sabin and Miller.<sup>8</sup> (i) Schulten.<sup>18d</sup> (j) Koller.<sup>17a</sup> (k) Scharf-Hansen.<sup>18i</sup>

59. Dameshek, W., and Valentine, E. H.: *Arch. Path.* **23**:159, 1937.

in turn proceed to form reticulocytes. Koller<sup>17a</sup> suggested that young multinucleated megaloblastic cells may subdivide and heteroplastically form a quantity of normoblasts. Limarzi and co-workers<sup>60</sup> also expressed the belief that since normal bipolar mitoses and maturation do not explain the rapid conversion of a megaloblastic marrow to a normoblastic one, multipolar mitoses and multinucleated erythroid cells, which are frequently seen in these marrows, may play a role. In even more recent studies Limarzi<sup>61</sup> attacked this problem by treating patients for pernicious anemia first with a solution of potassium arsenite (Fowler's solution) and then with potent liver extract. His tentative conclusion is that the arsenic-sensitive megaloblasts are not transformed into normoblasts.

In general, only patients having macrocytic anemia and megaloblastic marrow respond to specific anti-pernicious-anemia therapy. However, there have been rare patients with macrocytic anemia and genuine megaloblastic marrow who have failed completely to respond. This lack of response is thought to be due to failure of the marrow to utilize the specific active principle, or the marrow may have become exhausted.<sup>62</sup> On the other hand, patients with hyperplastic normoblastic or macro-normoblastic marrow do not as a rule respond to the administration of anti-pernicious-anemia principle, even though the marrow may show considerable erythroid immaturity. However, a favorable response has been reported in a few cases of macrocytic anemia accompanying cirrhosis of the liver.<sup>63</sup> Castle and Minot<sup>64</sup> obtained an irregular response in similar cases.

Recently, Segerdahl and Davidson and others<sup>65</sup> have reported that some women with normocytic hypochromic and microcytic hypochromic anemia of pregnancy and puerperium have megaloblastic marrow quite identical with that found in patients with Addisonian pernicious anemia at equivalent stages. These women responded to specific anti-pernicious-

60. Limarzi, Levinson and Jones.<sup>24d</sup> Limarzi, Jones and Levinson.<sup>24e</sup>

61. Limarzi, L. R.: *Proc. Central Soc. Clin. Research* **15**:12, 1942; *J. A. M. A.* **121**:1245, 1943.

62. (a) Wilkinson, J. F., and Israëls, M. C. G.: *Brit. M. J.* **1**:139 and 194, 1935. (b) Israëls, M. C. G., and Wilkinson, J. F.: *Quart. J. Med.* **5**:69, 1936. (c) Davidson, L. S. P.: *Edinburgh M. J.* **46**:474, 1939. (d) Wauchope, G. M., and Leslie-Smith, M.: *Lancet* **2**:1518, 1938. (e) Mahler, A., and Greenberg, D.: *J. A. M. A.* **112**:1150, 1939. Watson.<sup>38b</sup>

63. Wintrobe,<sup>19b</sup> Wintrobe, M. M.: *Arch. Int. Med.* **57**:289, 1936. Goldhamer, S. M.: *ibid.* **53**:54, 1934. Watson.<sup>17c</sup>

64. Castle, W. B., and Minot, G. R.: *Pathologic Physiology and Clinical Description of the Anemias*, New York, Oxford University Press, 1936.

65. (a) Segerdahl, E.: *Acta med. Scandinav.* **108**:483, 1941. (b) Davidson, L. S. P.; Davis, L. J., and Innes, J.: *Brit. M. J.* **2**:31, 1942. (c) Lambin, P., and de Weerd, W.: *Sang* **13**:929, 1939.

anemia therapy after a variable refractory period. Since patients with color indexes within or below the normal range responded to such therapy, the aforementioned observers<sup>65</sup> have suggested that "the institution of rational treatment is dependent upon sternal puncture." Such a condition is seemingly contradictory to the concept developed in this paper thus far. Fortunately, it has been my privilege to examine specimens of sternal marrow from similar patients with microcytic hypochromic anemia and megaloblastic marrow. This material will be described eventually in more detail by Wolff and Limarzi.<sup>15</sup> My interpretation is that the hypochromic anemia developed because of a cessation of activity on the part of the normoblastic series. The megaloblastic series had not developed sufficiently to produce enough acidophilic (orthochromic) megaloblasts to furnish megalocytes for distribution in the peripheral blood. The latter in sufficient numbers would have produced a macrocytic blood picture. Material of this type is excellent for demonstrating morphologic differences between the normoblastic and the megaloblastic series. The marrow of the aforementioned patients presumably occupies a position intermediate to the marrow described by Lambin and de Weerd<sup>65c</sup> in patients with very early pernicious anemia, in which megaloblasts were lacking, and that of patients with fully developed megaloblastosis of the marrow and macrocytic anemia. The fact that it responded to liver therapy emphasizes the need for recognizing genuine megaloblasts in bone marrow in order to determine the correct line of therapy. It is noteworthy that the pronounced development of the pathologic neutrophilic series (macropolyocytes) in the marrow was reflected in the peripheral blood even in the absence of macrocytic anemia. Wolff and Limarzi<sup>15</sup> have suggested that the presence of these atypical neutrophils in the blood might aid in separating this from other microcytic or normocytic types of hypochromic anemia.

Prior to the advent of liver therapy, arsenic was widely used in the treatment of pernicious anemia although no real benefit from its use could be proved.<sup>66a</sup> Evans<sup>66b</sup> considered that its use as a stimulant to hemopoiesis might be "whipping up" an already overactive marrow and thereby hastening its final collapse. But Naegeli<sup>15f</sup> (as late as 1935) still maintained that such therapy produced unquestionable remissions. It has been known for many years that arsenic acts as an irritant of marrow and as a protoplasmic poison. In studies on the thymus, lymph nodes, Peyer's patches, marrow, intestinal epithelium and testicular tissue of the mouse, Dustin<sup>67</sup> showed that nuclear chromatin

66. (a) Minot, G. R., and Lee, R. I.: Boston M. & S. J. **177**:761, 1917.  
(b) Evans, F. A.: Pernicious Anemia, Baltimore, Williams & Wilkins Company, 1926.

67. Dustin, A. P.: Strasbourg méd. **85**:12, 1927.

passes through certain phases during which it is hypersensitive to arsenic. This reaction (karyoclasia) takes place as the nucleus approaches a phase of condensation, either chromosomal or prepyknotic.

Tempka and Braun<sup>68</sup> observed the effect of arsenotherapy administered to a man with pernicious anemia for forty-five days. During this period he received daily 150 mg. of arsacetin (sodium acetylarsinate) by mouth and 5 mg. of arsenic acid subcutaneously for the first twenty-four days. Before this treatment the erythrocyte count was 1,250,000, the hemoglobin content 37 per cent, the leukocyte count 5,100 and the color index 1.5. After arsenotherapy the erythrocyte count was 3,210,000, the hemoglobin content 72 per cent, the leukocyte count 5,900 and the color index 1.1. The sternal marrow after seventeen days of arsenotherapy showed that while the promegaloblasts were unchanged, the other megaloblastic forms were somewhat reduced while the pronormoblasts and normoblasts were increased. The pathologic giant stab neutrophils, which are the precursors of the neutrophils in the peripheral blood of patients with pernicious anemia, were increased. Similar findings were present in the marrow forty-five days after the beginning of this therapy. From this it can be seen that arsenotherapy produces a slight remission in the peripheral blood by stimulating the marrow but that it does not influence qualitatively the pathologic composition of the marrow. Rohr<sup>16b</sup> reported the effects of arsenic on the marrow of 2 patients with pernicious anemia and illustrated in colors (fig. 17) many of the changes in megaloblasts. One patient who received 1 to 7 mg. of arsenic acid daily for seven days gave a negative response with an actual decrease in the number of erythrocytes and in the percentage of hemoglobin. Bone marrow aspirated on the seventh day after the commencement of therapy showed marked changes in the megaloblasts. Their nuclei were often swollen and misshapen. Extreme karyorrhexis (karyoclasia) with many bizarre nuclear protuberances was the commonest finding. Arsenotherapy was discontinued and injections of potent liver extract begun. Examination of the marrow eleven days later showed that the pathologic erythropoiesis had disappeared completely and that uniform macronormoblastic regeneration was established. Rohr<sup>16b</sup> summarized the effects of arsenic on the marrow in pernicious anemia by stating that it brings about pathologic stimulation of megaloblasts with hastening of cytoplasmic ripening, nuclear destruction and nuclear extrusion. This sometimes produces megalocytic remission in the blood but never normalization of the pathologic marrow. In other types of anemia arsenic stimulates normal erythropoiesis. Koller<sup>17a</sup> estimated the length of time it takes for basophilic megaloblasts to mature to megalocytes by studying sternal marrow from a patient with

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68. Tempka, T., and Braun, B.: *Folia haemat.* 48:355, 1932.

pernicious anemia (case 42) who was treated with 10 daily doses of arsenic acid (2 to 10 mg.). The marrow was examined before therapy and six and ten days afterward. On the sixth day polychromatophilic megaloblasts were more than doubled in number. On the tenth day acidophilic (orthochromatic) megaloblasts were increased about eleven times. From these data he calculated that it takes ten to twelve days for megalocytes to mature, in contrast to the three to four days for normocytes, following liver therapy. Koller was not unmindful of the fact that arsenic medication is unphysiologic.

In a preliminary report, Limarzi and associates<sup>69</sup> substantiated Rohr's findings in the marrow of pernicious anemia following arsenotherapy. They also studied marrow from patients with sickle cell anemia, erythroblastosis, hypochromic anemia, cirrhosis of the liver, carcinoma of the stomach, polycythemia vera and normal controls following oral administration of a solution of potassium arsenite (Fowler's solution). The striking thing was the inability of the drug to produce as extensive or corresponding changes in the normoblastic cells, even though some marrows exhibited marked erythroid immaturity. In cases of spherocytic hemolytic jaundice and 1 case of refractory anemia with microcytosis the administration of arsenic produced karyorrhexis in polychromatic and acidophilic normoblasts. However, in all instances the pronormoblasts remained intact. Hence, these findings have been interpreted as additional evidence for the separation of the pathologic megaloblastic series from the normoblastic series of red cell regeneration.

#### COMMENT

When Doan, Cunningham and Sabin<sup>1</sup> were formulating their theory of erythropoiesis, they placed more emphasis on the physiologic than on the morphologic approach, primarily because Sabin was of the opinion that these problems had passed beyond the morphologic stage.<sup>70</sup> Sabin was impressed by Naegeli's classification of leukocytes because it was functional and could be used in the clinical study of blood.<sup>71</sup> Using the same line of reasoning for the red blood corpuscles, one finds it most peculiar that Sabin did not accept any of Naegeli's views<sup>72</sup> regarding erythropoiesis, for the recognition of a megaloblastic and a normoblastic series of red cell development certainly has a functional basis.

69. (a) Limarzi.<sup>61</sup> (b) Limarzi, L. R., and Levinson, S. A.: *Proc. Inst. Med. Chicago* **14**:232, 1942. (c) It has been my privilege to examine many of Dr. Limarzi's preparations and verify his interpretation of the effect of arsenic on megaloblasts.

70. Sabin, F. R.: (a) *Physiol. Rev.* **2**:38, 1922; (b) **8**:191, 1928.

71. Sabin,<sup>70a</sup> p. 44.

72. Naegeli, O.: *Blutkrankheiten und Blutdiagnostik*, ed. 4, Berlin, Julius Springer, 1923.



From a purely physiologic point of view the youngest hemoglobiniferous cells under embryonic, normal adult and pathologic conditions have only one function to perform, which is to begin synthesizing hemoglobin. There is, however, some evidence to indicate that in some of these conditions hemoglobin may differ.<sup>73</sup> Moreover, from the aforementioned distinction of megaloblasts it should be realized that these cells and their products are functionally as well as structurally abnormal, so that they are not just "the same old red cell" under different conditions.

It is possible to distinguish morphologically between the early hemoglobiniferous cells under certain conditions. The differences in nuclear structure between the primitive erythroblasts of the embryo, the promegaloblasts of pernicious anemia and the pronormoblasts (proerythroblasts) of normal marrow may be due to differences in extracellular environment, in intracellular metabolism, in the processes of the synthesis of hemoglobin or in a combination of these. Although the differences in nuclear structure seem insignificant to those who are unaccustomed to making a detailed examination of the nucleus, they are constant and appear under entirely different conditions. These cells should not be grouped together under one heading according to the scheme of Doan, Cunningham and Sabin<sup>1</sup> or that of Davidson, Davis and Innes<sup>74</sup> for at least two reasons: First, it would be impossible to determine the factors governing these differences in nuclear structure under various conditions. Second, a valuable morphologic diagnostic criterion would be buried.

Regardless of the controversy over the origin of megaloblasts and the fate of these cells following liver therapy, the fact remains that there is no argument among the majority of European clinical pathologists concerning the importance of recognizing and the implications of finding true megaloblastosis of the marrow. Furthermore, complications such as cancer, syphilis, diabetes mellitus, nephritis and hyperthyroidism apparently do not disturb this characteristic pattern of marrow in untreated pernicious anemia.<sup>75</sup> The ability to recognize and separate megaloblastic marrow from other erythroblastic marrows, such as those encountered in the anemias accompanying hepatic cirrhosis, gastric carcinoma and various hemolytic processes, should be cultivated and developed among students of clinical pathology in this country. However, this refinement of morphologic hematology will be retarded if clinical pathologists accept the claim of Sabin and Miller<sup>8</sup> that the marrow from a 2 week old rabbit is "as instructive for the study of maturation as the blood of a patient with pernicious anemia for the red series, or with myelogenous leucemia for the white cells." This holds true only for the progressive changes

73. Brinkman, R., and Jonxis, J. H. P.: *J. Physiol.* **85**:117, 1935.

74. Davidson, L. S. P.; Davis, L. J., and Innes, J.: *Quart. J. Med.* **11**:19, 1942.

75. Limarzi, L. R.: *Illinois M. J.* **75**:38, 1939.

from a basophilic to an acidophilic cytoplasm and not for the nuclear structure, since the latter is entirely different in the two situations.<sup>76</sup> It cannot be emphasized too strongly that the marrow from a 2 week old rabbit should not be used as classroom material to teach the important differential diagnostic characteristics of developing red cells in pernicious anemia.

As mentioned previously, Sabin and Miller<sup>8</sup> expressed the opinion that the youngest red cells in normal rabbit marrow are of the same type as the most immature erythroid forms seen in the blood in pernicious anemia. They show, in their figure 5, of a dry fixed smear, a cell labeled *Mg*, which is supposed to represent a megaloblast in marrow from a normal 5 day old rabbit. The nuclear structure is definitely like that of cells which have been described by Pappenheim, Naegeli, Ferrata, Maximow, Michels, Fieschi, Rohr, Schulten, Scott, Dameshek and Jones as belonging to the definitive or normoblastic series. As a matter of fact, it is a poor selection of a cell type which is to represent the earliest stage of any erythroid series since the nuclear structure is entirely too pachychromatic. If such a cell is compared with primitive erythroblasts of the rabbit yolk sac and with megaloblasts from the marrow of pernicious anemia during severe relapse, it is obvious that there is no morphologic justification for Sabin and Miller designating it as a "megaloblast." I should mention that after a demonstration of marrows from patients with pernicious anemia and normal rabbits at the meetings of the anatomists at the University of Pittsburgh (1938), Sabin finally conceded that cells morphologically identical with megaloblasts are not present in normal rabbit marrow.

Megaloblastosis of the marrow produces an increase of the mean corpuscular diameter and of the volume of the red cells in the peripheral blood. This megalocytic anemia is macrocytic, but it does not follow that all macrocytic types of anemia are necessarily megalocytic.<sup>77</sup> Therefore, it would be unjustifiable to deduce the presence of megaloblasts in the hemopoietic organs or the qualitative identity of blood pictures from peripheral macrocytosis alone.<sup>78</sup> Israëls<sup>79</sup> mentioned that certain types of hemolytic anemia and those accompanying carcinoma of the stomach have been assumed to possess megaloblastic erythropoiesis just because the Price-Jones curve has shown an increase in the cell diameters. However, no megaloblasts were present in the marrow.

76. Jones.<sup>18a</sup> Jones.<sup>54j</sup> Kirschbaum.<sup>30</sup>

77. (a) Davidson.<sup>62c</sup> (b) Israëls, M. C. G.: *J. Path. & Bact.* **49**:231, 1939. (c) Wilson.<sup>17b</sup> (d) Scott.<sup>53</sup> (e) Jones.<sup>54j</sup> (f) McGowan, J. P.: *Edinburgh M. J.* **49**:568, 1942.

78. Davidson.<sup>62c</sup> Israëls.<sup>77b</sup> Jones.<sup>54j</sup>.

79. (a) Israëls.<sup>77b</sup> (b) Israëls, M. C. G.: *J. Path. & Bact.* **52**:361, 1941. (c) Israëls.<sup>54k</sup>

Many macrocytic types of anemia are not related to a deficiency of liver principle of the addisonian or the pernicious type. As a group the macrocytic types are very heterogeneous, with perhaps as many different causes.<sup>80</sup> The diversity of this group can be illustrated by a list of conditions in which at some time during their course macrocytosis occurs. They are: Hodgkin's disease, aplastic or hyporegenerative anemia, multiple myeloma, carcinoma of the stomach, sigmoid colon and rectum, leukemia, pellagra, myxedema, lead poisoning, congenital and tertiary syphilis, metastatic tumors, malignant malaria, acquired or secondary acute hemolytic anemia, hepatic and splenic disease, acute rheumatic fever, erythroblastosis foetalis and reticuloendotheliosis.<sup>81</sup>

There are five fundamental cellular causes for an increase in the mean corpuscular diameter of red cells. They may produce macrocytosis either independently or, in some cases, in combination with one another. From a practical point of view, clinical pathologists should be able to recognize the first three of these fundamental causes of macrocytosis and be aware of the existence of the fourth. The first two fundamental causes of macrocytosis differ from each other primarily in size relationships. The third and fourth show differences in the origin and the maturation of the cells involved, whereas the fifth differs from all of the rest not only in the mode of origin and of maturation of the cells but also in that it is limited to the prehepatic period of embryonic development.

The first cause of macrocytosis in the peripheral blood may be a marked outpouring of reticulocytes from normal marrow. The macrocytosis here is due to the fact that reticulocytes or immature erythrocytes are larger than nonreticulated mature ones.<sup>82</sup> In cases of posthemorrhagic anemia, if the stimulation of the marrow has been great, a slight increase in the mean corpuscular volume may be present during the period of maximal reticulocytosis.<sup>83</sup>

The second cellular mechanism by which macrocytosis may develop is the presence of macronormoblastic marrow. This differs from normal marrow in that it is hyperplastic, the normoblasts (erythroblasts) are larger and there are more of the early forms (pronormoblasts and basophilic normoblasts). In general, this type of marrow is the one found in the macrocytic types of anemia not related to a deficiency of

80. Fallon.<sup>19c</sup> Davidson.<sup>62c</sup> Wintrobe.<sup>19b</sup> Wilson.<sup>17b</sup>

81. (a) Fallon.<sup>19c</sup> (b) Davidson.<sup>62c</sup> (c) Israëls.<sup>77b</sup> (d) Watson, C. J.: *Ann. Int. Med.* **12**:1782, 1939. (e) Watson, C. J., and Clarke, W. O.: *Bull. Univ. Minnesota Hosp.* **12**:356, 1940. (f) Dameshek, W., and Schwartz, S. O.: *Medicine* **19**:231, 1940. (g) Wilson.<sup>17b</sup> (h) Limarzi.<sup>15</sup> (i) Reisner, E. H.: *Arch. Int. Med.* **71**:230, 1943.

82. Isaacs.<sup>2</sup> Wintrobe.<sup>19b</sup>

83. (a) Wintrobe.<sup>19b</sup> (b) Wintrobe, M. M.: *J. Clin. Investigation* **13**:669, 1934.

liver principle. The large pronormoblasts present in these hyperplastic marrows may be the cells which some authors have misinterpreted as megaloblasts. However, careful examination will show that the resemblance is only superficial.<sup>84</sup> Another stumbling block may be due to the presence of myeloblasts with a very basophilic cytoplasm.<sup>5</sup> Dameshek and Schwartz<sup>81f</sup> measured the diameter of these nucleated red cells in the marrow from a patient with acute hemolytic anemia and found the mean diameter to be 1.0 to 1.2 microns larger than normal. The nuclear structure of the cells in macronormoblastic marrow is essentially the same as that in normal marrow with the exception that the nuclei are slightly larger. Not only are the reticulocytes larger but the mature nonreticulated corpuscles are larger than normal and hence can be properly designated as macrocytes. Consequently, it is possible to have macrocytic anemia which is not correlated with an increase in reticulocytes.<sup>84a</sup>

One of the best sources of material in which to study the structure of macronormoblasts is a dry smear or imprint of fetal liver.<sup>85</sup> Contrary to Dameshek and Valentine<sup>59</sup> and others,<sup>86</sup> megaloblasts are not present there. Recently Wilson<sup>17b</sup> erroneously reported young primitive erythroblasts present in fetal livers. The only primitive erythroblasts present in fetal livers are the late or acidophilic forms, which have been derived from mesenchymal precursors in the embryonic yolk sac.<sup>87</sup>

It is generally agreed that any process which interferes with the production, absorption, utilization or storage of the anti-pernicious-anemia principle will result in the production of megaloblastic marrow. Theoretically, patients with hepatic cirrhosis might be expected to show such alteration of marrow since it has been postulated that the storage of the liver principle is impaired in this disease. On the contrary, it has been the repeated experience of many hematologists that in the presence of cirrhosis of the liver the marrow is macronormoblastic in type.<sup>88</sup> As a matter of record, Israëls and Wilkinson<sup>89</sup> placed special emphasis on the

84. Dameshek and Schwartz.<sup>81f</sup> Wilson.<sup>17b</sup> Jones.<sup>5</sup>

84a. Watson and Clarke.<sup>81e</sup> Reiser.<sup>81i</sup> Watson.<sup>17c</sup>

85. Jones.<sup>18a</sup> Jones.<sup>21c</sup> Kirschbaum.<sup>30</sup>

86. (a) Aron, M.: *Arch. de morphol. gén. et expér.*, 1922, no. 10. (b) Zanaty, A. F.: *Virchows Arch. f. path. Anat.* **293**:794, 1934. (c) Gilmour, J. R.: *J. Path. & Bact.* **52**:25, 1941.

87. (a) Kirschbaum.<sup>30</sup> (b) Bloom.<sup>12</sup> (c) Bloom, W., and Bartelmez, G. W.: *Am. J. Anat.* **67**:21, 1940.

88. (a) Limarzi.<sup>75</sup> (b) v. d. Merwe, C. F.: *Folia haemat.* **55**:218, 1936. (c) Rohr.<sup>16</sup> (d) Scott.<sup>58</sup> (e) Dameshek and Schwartz.<sup>81f</sup> (f) Wilson.<sup>17b</sup> (g) Benhamou, E.: *Presse méd.* **47**:755, 1939. (h) Markoff, N.: *Deutsches Arch. f. klin. Med.* **183**:289, 1938. (i) Revol, L.: *L'exploration de la moelle osseuse par ponction sternale*, Paris, J. B. Ballière et fils, 1938. (j) Dreyfus, C.: *Schweiz. med. Wchnschr.* **71**:682, 1941. (k) Jones.<sup>5</sup> Israëls.<sup>54k</sup>

89. Israëls, M. C. G., and Wilkinson, J. F.: *Quart. J. Med.* **9**:163, 1940.

fact that they have yet to see a case of hepatic cirrhosis in which megaloblastic marrow accompanied macrocytosis in the peripheral blood. Likewise, the absence of dysplastic marrow in cases of gastric carcinoma has been used to separate the anemia in this disease from true pernicious anemia.<sup>90</sup> However, the coincidence of pernicious anemia and gastric carcinoma, or the later development of gastric carcinoma in a patient with pernicious anemia, complicates this problem.

Doan,<sup>58a</sup> Sabin<sup>1</sup> and others<sup>91</sup> have maintained that megaloblasts may become more and more prominent as the demand on the blood-forming tissues increases. If this were so, marrow from patients with hemolytic anemia should have an increased number of these cells, especially during the period of a crisis. Here again, practically all hematologists are agreed that the marrow is hyperplastic and normoblastic or macronormoblastic in cellular composition.<sup>92</sup> In a few instances the pronormoblasts are atypical and arise directly from the reticulum,<sup>93</sup> which is very likely why Tötterman<sup>94</sup> mistook them for megaloblasts.

Dameshek and Schwartz<sup>81f</sup> made it a point to emphasize that the macrocytic anemia accompanying hemolytic processes is not due to the presence of orthochromatic macrocytes such as those seen in pernicious anemia. They suggested that this type of anemia should be classified as pseudomacrocytic since the macrocytosis is due to the presence of a large number of polychromatophilic reticulocytes. This does not seem to be warranted since "macrocytic" is a general term designating a quantitative attribute of the blood with no regard for the qualitative nature of the cells involved.

A megalocytic hypochromic anemia of pancreatic disease was reported by Cheney.<sup>95a</sup> Postmortem examination of marrow in 2 of the 6 cases revealed it to be hyperplastic and normoblastic (erythroblastic). Consequently the mechanism responsible for the increased corpuscular diameter was not a megaloblastic marrow as implied by the title of the article. Similarly, the macrocytic anemia in patients with erythroblastosis foetalis is not due to megaloblastosis as reported by Reisner.<sup>81i</sup> The large cells in his figure 11 have a nuclear pattern quite characteristic for cells in early stages of normoblastic or macronormoblastic

90. Wilkinson, J. F.: *Acta med. Scandinav.* **80**:466, 1933. Limarzi.<sup>75</sup> Merwe.<sup>88b</sup> Israëls.<sup>77b</sup> Rohr.<sup>16</sup> Jones.<sup>5</sup>

91. Steele, B. F.: *J. Exper. Med.* **57**:881, 1933. Tocantins, L. M.: *Medicine* **17**:155, 1938. Osgood.<sup>3</sup> Isaacs.<sup>2</sup>

92. Davidson.<sup>92c</sup> Israëls.<sup>77b</sup> Limarzi.<sup>75</sup> Dameshek and Schwartz.<sup>81f</sup> Israëls.<sup>79b</sup> Davidson, Davis and Innes.<sup>74</sup> Scott.<sup>53</sup> Wilson.<sup>17b</sup> Henning.<sup>58b</sup> Rohr.<sup>16</sup> Fieschi.<sup>18c</sup> Israëls.<sup>54k</sup>

93. Jones.<sup>21b</sup> Jones.<sup>18a</sup>

94. Tötterman, G.: *Acta med. Scandinav.* **90**:527, 1936.

95. (a) Cheney, G.: *Folia haemat.* **56**:28, 1936. (b) Anderson, G. W.: *Am. J. Obst. & Gynec.* **42**:1, 1941.

development. Even though Reisner has rearranged and modified the nomenclature used by Dameshek and Valentine<sup>59</sup> and Jones,<sup>18a</sup> those cells are certainly not genuine megaloblasts. This is further substantiated by the fact that my examination of material used by Dr. George W. Anderson in his study of nucleated red cell counts of fetal blood and blood of newborn infants failed to reveal the presence of megaloblasts.<sup>95b</sup>

The third cellular mechanism which produces macrocytosis in the peripheral blood is the megaloblastic marrow. Certain it is, however, that precocious hemoglobinization of erythroblasts in hyperplastic marrow should not be used as a criterion for the identification of megaloblasts.<sup>96</sup> This is admirably illustrated in a study of hemolytic anemia by Israëls,<sup>97</sup> in which he also emphasized that fine nuclear distinctions are lost in histologic sections. Furthermore, if Gilmour and Turnbull<sup>96</sup> were correct about megaloblasts being normal cells which have undergone precocious hemoglobinization, the youngest red cells in hyperplastic erythroblastic marrow should show the same sensitivity to arsenic as megaloblasts in pernicious anemia during relapse. This was not the case in the recent studies reported by Limarzi.<sup>61</sup> In all instances the pronormoblasts of such marrow were resistant to arsenic.

Although megaloblastic marrow is most frequently found in patients with anemia due to deficiency of liver principle, it must be admitted that it has been reported in totally unrelated conditions by authors who apparently were acquainted with the nuclear structure of genuine megaloblasts. The conditions in which these megaloblasts have been reported are: atypical myelosis with intense erythroblastosis; erythropoietic regeneration following irradiation of a cancer; localized erythropoietic hyperplasia in roentgen aleukia<sup>16a</sup>; erythroleukemia<sup>18j</sup>; monocytic leukemia<sup>79b</sup>; generalized tuberculosis<sup>88c</sup>; severe sepsis<sup>68</sup>; agranulocytosis<sup>98</sup>; leishmaniasis,<sup>99</sup> and carcinoma of the esophagus.<sup>17b</sup> Dameshek and Valentine<sup>59</sup> suggested that this sporadic occurrence of megaloblasts may be due to an unrecognized deficiency of liver principle. It is evident that megaloblasts alone are not pathognomonic for pernicious and related types of anemia, assuming that in the cases just mentioned there was no difference in the criteria for cellular identification.

It may be established eventually that the presence of genuine megaloblasts together with the giant stab form neutrophils, which are a part of the pathologic neutrophilic series,<sup>100</sup> is pathognomonic for this type of

96. Gilmour.<sup>86c</sup> Turnbull, H. M., in Vaughan, J. M.: *The Anemias*, London, Oxford University Press, 1934.

97. Israëls, M. C. G.: *J. Path. & Bact.* **52**:361, 1941.

98. Watson.<sup>17c</sup> Jones.<sup>18a</sup> Dameshek, W., and Ingall, M.: *Am. J. M. Sc.* **181**:502, 1931.

99. Kassirsky, I. A.: *Folia haemat.* **51**:352, 1934.

100. Jones.<sup>21d</sup> Jones.<sup>35b</sup> Jones.<sup>18a</sup>

macrocytic anemia. The presence of a pathologic neutrophilic series in the marrow of pernicious anemia is further indirect evidence to support my contention that the megaloblastic series is pathologic and represents neither a return to embryonic conditions nor an arrest of maturation in cells normally present in the marrow of adults.<sup>101</sup> Nordenson<sup>52</sup> and Henning<sup>58b</sup> claimed that abnormal neutrophils alone are pathognomonic of pernicious anemia. Wilson<sup>17b</sup> encountered giant stab form neutrophils only in pernicious anemia. In my material they have been found in marrow from patients with pernicious anemia, achrestic anemia, tropical macrocytic anemia, tropical sprue and pernicious anemia of pregnancy.<sup>102</sup> It is likely many American hematologists have not paid sufficient attention to alterations in the neutrophilic series and especially to the pan-myelopathy which exists in the marrow of pernicious anemia during relapse.<sup>100</sup>

In considering this third mechanism which may produce a macrocytic blood picture, it should be realized that the megaloblastic series produces reticulated megalocytes as well as nonreticulated ones.<sup>6b</sup> The origin of poikilocytes will not be discussed here, since it has recently been considered elsewhere.<sup>103</sup>

The fourth cellular mechanism which may produce a macrocytic blood picture is an extremely rare condition, the occurrence of which has not been accepted generally nor has a sufficient number of cases been encountered to permit extensive study. Downey<sup>104</sup> and his students recognized a morbid condition that is characterized hematologically and histologically by irreversible hyperplasia of the reticulum with concomitant cellular differentiation of the liberated cells toward the hemoglobiniferous series and their circulation in the blood. This specific condition is one of the variants of leukemic reticuloendotheliosis.<sup>104</sup> In this disease the youngest nucleated red cells exhibit a nuclear structure which resembles that of the undifferentiated reticulum cells seen in dry smear preparations of hemopoietic organs. This reticular characteristic of the nucleus persists even though the cells are quite acidophilic. The final stage of this type of erythropoiesis is not unlike that of other erythroblasts in that the nucleus becomes pyknotic. This leukemic type

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101. Jones,<sup>100</sup> Wilson,<sup>17b</sup> Rohr,<sup>16</sup> Fieschi,<sup>18c</sup>

102. Some of these marrows were furnished by the following men: Dr. L. R. Limarzi (pernicious anemia and pernicious anemia of pregnancy), the late Dr. I. J. Pass and Dr. C. J. Watson (achrestic anemia), Dr. W. M. James, Panama, Republic of Panama (tropical macrocytic anemia), Dr. R. Rodriguez-Molina, Puerto Rico (tropical sprue).

103. Tischendorf,<sup>21f</sup> Schultz, W., and Buding, A.: *Deutsche med. Wchnschr.* 66:492, 1940.

104. Downey, H.: *Monocytic Leucemia and Leukemic Reticulo-Endotheliosis*, in Downey, H.: *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 1321, case 7.

of erythropoiesis differs from the reticular origin of megaloblasts and pronormoblasts<sup>93</sup> in that the reticular nuclear pattern is not limited to a single stage but may be found in the basophilic, polychromatophilic and acidophilic (orthochromatic) stages. These cells have skipped the myeloblast stage during their development from the reticulum.<sup>104</sup> This leukemic type of erythropoiesis also differs from the reticular origin of megaloblasts and pronormoblasts of other types of anemia in that it is an irreversible cellular change which has not been influenced by any known therapy.

In a case of my collection the non-nucleated forms were definitely macrocytic.<sup>5</sup> In the case reported by Limarzi and associates<sup>105</sup> the abnormal cells resembled megaloblasts more closely than they did cells of any other type with the possible exception that they might have been classed as a veritable erythroblastoma. The multinucleated giant erythroblasts formed non-nucleated corpuscles as much as 21 by 26 microns in diameter. To those accustomed to making a critical analysis of nuclear structure these leukemic cells are not genuine megaloblasts nor are they macronormoblasts. It is felt that the red cells in the case of "acute megaloblastic leukemia" described by Penati<sup>106</sup> really belong to this group, which may explain why it was so difficult to classify the cells.

The fifth cellular mechanism which aids in the production of a macrocytic blood picture is the development of the primitive erythroblastic series in the yolk sac of mammalian and avian embryos during the pre-hepatic period. These cells arise directly from mesenchymal elements of the yolk sac and are not formed in the fetal liver and spleen, nor in the adult bone marrow.<sup>107</sup> The megaloblasts of pernicious anemia are similar to but not identical with the primitive erythroblasts of the embryo.<sup>76</sup> The large non-nucleated products of this series circulate in the peripheral blood up to the time of birth in animals having a short period of gestation.<sup>30</sup> On the other hand, these cells disappear from the blood of human and pig fetuses a considerable time before birth.<sup>30</sup>

With these various morphologic mechanisms in mind it is possible to analyze qualitatively various blood pictures which have been grouped under one heading merely because they possess one quantitative attribute, namely, macrocytosis. It is not the purpose of this review to carry the analysis to completion but only to indicate a possible trend for future qualitative studies of macrocytic blood pictures.

The macrocytic blood picture in the late stages of human and pig fetuses, studied by Wintrobe and Shumacker,<sup>108</sup> is not produced by

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105. Limarzi, Levinson and Jones.<sup>24d</sup> Limarzi, Jones and Levinson.<sup>24e</sup> Dr. Limarzi sent me duplicate bone marrow preparations from this case.

106. Penati, F.: *Minerva med.* 2:401, 1937.

107. Kirschbaum.<sup>30</sup> Bloom and Bartelmez.<sup>87c</sup> Bloom.<sup>12</sup>

108. Wintrobe, M. M., and Schumacker, H. B.: *J. Clin. Investigation* 14:837, 1935.



megalocytosis similar to that seen in persons with pernicious anemia, since megaloblasts are not present in the fetal liver and spleen.<sup>76</sup> If this blood picture is likened to that of any type of macrocytic anemia, it should be that seen in cases of hepatic cirrhosis in which there is a macro-normoblastic marrow.<sup>88</sup> However, when it is considered that the fetal macronormoblasts are formed in the liver, whereas those in the cirrhotic patient are formed in the marrow, this similarity is not so simple.

The blood of the newborn rat has been used in the study of the effect of antianemic substances administered during gestation just because there is a quantitative similarity to blood from patients with pernicious anemia.<sup>109</sup> When the cellular mechanisms responsible for these two macrocytic blood pictures are studied, it is found that the two bloods are qualitatively quite different. For example, the macrocytic blood picture in pernicious anemia during severe relapse is produced by the products of the pathologic megaloblastic series, namely, the megalocytes and a few reticulated megalocytes. On the other hand, when the blood of the newborn rat is analyzed qualitatively, it is found that three different mechanisms are responsible for the final picture of macrocytosis.<sup>54j</sup> First, there are 90 per cent or more reticulocytes. The larger ones can be traced back to an origin from macronormoblasts in the liver, and the others have come from normoblasts in the marrow. The newborn rat also has a few cells circulating in its blood which are the end products of those originally formed intravascularly in the yolk sac during the pre-hepatic period.

When the increase in mean corpuscular volume<sup>83</sup> that occurs during liver therapy in cases of pernicious anemia is analyzed, it is found to be due to two different mechanisms.<sup>6b</sup> At the peak of a reticulocyte response there is an outpouring of immature red corpuscles, but this response is qualitatively different from other reticulocyte responses.<sup>6b</sup> It is composed of normal reticulocytes, reticulated megalocytes and mature megalocytes. The latter really represent the result of the purging of the marrow of its pathologic elements.<sup>110</sup> Mustafa<sup>54b</sup> measured cell diameters before and after liver therapy. At the height of the reticulocyte crisis he obtained a double peak curve, which has been interpreted to indicate an outpouring of megalocytes. Evidence to support my contention that the reticulocyte response in pernicious anemia is different from others<sup>6b</sup> is found in the studies by Cotti and others<sup>111</sup> on the uric acid output during this period.

In studying macrocytic blood pictures in man under various pathologic conditions, considerable attention should be directed to the qualita-

109. Jones,<sup>54j</sup> pp. 481-483.

110. Jones,<sup>56b</sup> Tischendorf,<sup>21f</sup> Mustafa,<sup>54b</sup> Stasney, J., and Pizzolato, P.: *Proc. Soc. Exper. Biol. & Med.* **51**:335, 1942.

111. Cotti, L., and Balestrieri, F.: *Gior. di clin. med.* **20**:628, 1939. Stasney, J., and Pizzolato, P.: *Proc. Soc. Exper. Biol. & Med.* **51**:338, 1942.

tive analysis of the marrow cells as seen in dry smears. It should be realized that although there are four cellular mechanisms, each of which may produce a macrocytic blood picture, normoblastic erythropoiesis is always present to a variable extent. For example, even though the dominant type of erythropoiesis in the marrow in pernicious anemia is of the megaloblastic type, normoblasts are present. The latter may be dormant or slightly active. Lambin and de Weerd<sup>18j</sup> and Schartum-Hansen<sup>18i</sup> postulated an intermediate form of erythroblast in pernicious anemia. That has not been necessary in my experience nor in that of Israëls.<sup>77b</sup> In hepatic cirrhosis, macronormoblasts dominate the picture, but there is a fairly good percentage of active normoblasts. The latter are also quite active in the hemolytic anemias. In leukemic reticulo-endotheliosis<sup>105</sup> the giant bizarre multinucleated erythroblasts were the dominant feature, but the normoblastic series was proliferating quite actively.

From the foregoing review it should be evident that the megaloblast-normoblast problem is not merely a quibbling over the minutiae of terminology. To be sure, terminology has been confused. But to dismiss this whole problem of erythropoiesis by saying that it is entirely one of terminology is to admit a lack of knowledge concerning fundamental problems of erythropoiesis. The whole problem centers around the ability to recognize and appreciate differences in nuclear structure, the ability to separate pathologic from normal cells and the ability to separate normal or hyperplastic marrow patterns from dysplastic ones. These distinctions can be accomplished if good, fresh and well stained dry smears are studied. This technic brings out the delicate nuclear structure necessary if one is to discriminate between normal and pathologic erythropoietic cells. This cannot be done with the supravital technic. As Schwind<sup>37</sup> has pointed out, the latter is suited for a study of the cytoplasm, and there are no striking differences in the cytoplasm of the megaloblastic and normoblastic series, as there are in the nuclei.

Medical students should not be taught that the dominant type of erythropoietic cell seen in the marrow of pernicious anemia during relapse is present in normal marrow or that it has been readily produced experimentally (with one possible exception<sup>112</sup>), or that it increases with increasing demands on the blood-forming organs.

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112. Wills, L., and Stewart, A.: *Brit. J. Exper. Path.* **16**:444, 1935.

# Forensic Medicine

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## DIFFERENTIATION OF FETAL AND ADULT HUMAN HEMOGLOBIN

ITS MEDICOLEGAL IMPORTANCE, ESPECIALLY IN CONNECTION WITH  
THE ALKALI TEST FOR CARBON MONOXIDE IN BLOOD

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That the hemoglobin of most animals is more resistant than human hemoglobin to the denaturizing action of alkalis was discovered in 1866 by Koerber.<sup>1</sup> His observations were confirmed by Krueger<sup>2</sup> and by Ziemke,<sup>3</sup> the latter making use of this discovery to differentiate human from animal blood. Bischoff<sup>4</sup> later found that hemoglobin from the human fetus resembled animal hemoglobin in that it was also strongly resistant to the denaturizing action of alkali and that it was unlike human adult hemoglobin, which is rapidly changed into alkaline hematin on the addition of alkali. Koerber<sup>1</sup> had already observed that human placental blood was more alkali resistant than adult blood.

Various methods may be employed to demonstrate the difference between fetal and adult hemoglobin in resistance to the action of alkali. The simplest method, that of Krueger,<sup>2</sup> can be carried out with a hand spectroscope. This method was used by Bischoff<sup>4</sup> and Trought.<sup>5</sup> More exact results may be obtained by the more complicated spectrophotometric method mentioned by Haurowitz<sup>6</sup> or by the photo-electric method used by Brinkman and his co-workers.<sup>7</sup> Recently,

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1. Koerber, E.: Ueber Differenzen des Blutfarbstoffs, Inaug. Dissert., Dorpat, 1866; cited by Ziemke.<sup>3</sup>

2. Krueger, F.: *Ztschr. f. Biol.* **24**:314, 1888; *Ztschr. f. vergl. Physiol.* **2**:254, 1925.

3. Ziemke, E.: *Vrtljschr. f. gerichtl. Med.* **22**:77, 1901.

4. Bischoff, H.: *Ztschr. f. d. ges. exper. Med.* **48**:472, 1926. Bischoff, F., and Schulte, H.: *Jahrb. f. Kinderh.* **112**:56, 1926.

5. Trought, H.: *Arch. Dis. Childhood* **7**:259, 1926.

6. Haurowitz, F.: *Ztschr. f. physiol. Chem.* **183**:78, 1929; *Ztschr. f. physiol. Med.* **186**:141, 1930.

7. Brinkman, R.; Wildschut, A., and Witterman, A.: *J. Physiol.* **80**:377, 1934. Brinkman, R., and Jonxis, I. H. P.: *ibid.* **85**:117, 1935.

Darrow, Nowakowsky and Austin<sup>8</sup> prepared specific rabbit antisera which react with and differentiate between human adult and fetal hemoglobin.

Fetal and adult hemoglobin differ in reaction to alkali because of the presence in fetal blood of two kinds of hemoglobin, one resistant and the other nonresistant to alkali. Haurowitz estimated that the blood of the newborn infant contains 80 per cent of resistant and 20 per cent of nonresistant hemoglobin. The amount of resistant hemoglobin diminishes gradually after birth; Brinkman and his co-workers found that it had disappeared entirely from the blood of infants 6 to 7 months of age. Trought stated that the resistant form of hemoglobin may disappear as early as four and a half months after birth, and Bischoff observed that it disappeared more rapidly from the blood of a full term baby than from that of a premature one. After the disappearance of the resistant form the hemoglobin of an infant cannot be differentiated from that of an adult.

The Krueger method for determining the resistance of hemoglobin to alkali is carried out in the following way:

One cubic centimeter of a 1 per cent solution of sodium hydroxide is added to 5 cc. of an approximately 1 per cent solution of blood in a test tube. The untreated dilute blood solution is pinkish red, and when it is examined through the spectroscope, the two absorption bands of the oxyhemoglobin spectrum between D and E are plainly visible. The alkali which is added denatures the hemoglobin, changing its red color into the brown color of alkaline hematin and causing a disappearance of the two bands of the oxyhemoglobin spectrum. The denaturation time for the hemoglobin of adults or of infants 6 months of age or older is about one minute; the maximum, five minutes. The denaturation time for the hemoglobin of a fetus or a newborn infant is never less than one hour and more often two to three hours or longer. In many fetal bloods we have observed the absence of denaturation by alkali after twenty-four hours. The hemoglobin of infants from a few weeks to 3 months of age also exhibits a longer denaturation time than that of adults, the process requiring from one to two hours. The denaturation of adult hemoglobin into alkaline hematin is delayed slightly if a more concentrated solution of blood is used, but not sufficiently to confuse the results. The striking difference in resistance to the action of alkali between adult and fetal hemoglobin is evident even if more concentrated blood solutions are used for testing.

The alkaline hematin which is formed slowly from fetal hemoglobin is similar to that which is formed rapidly from adult hemoglobin in that it is transformed rapidly into hemochromogen on the addition of a few drops of dilute ammonium sulfide solution.

The difference between human adult and fetal hemoglobin in resistance to a dilute alkaline solution has considerable medicolegal importance, as this test may be used to differentiate between adult and fetal human blood stains. The alkali-resistant property of fetal hemoglobin inter-

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8. Darrow, R.; Nowakowsky, S., and Austin, W.: *Arch. Path.* **30**:873, 1941.

feres with the commonly used test for the detection of carbon monoxide in blood, which is based on the resistance of carboxyhemoglobin to the action of alkali, in contrast to oxyhemoglobin. Obviously, the use of this test on the blood of a newborn or a very young infant might lead to a false positive result.

#### INTERFERENCE WITH TESTS FOR DETECTION OF CARBON MONOXIDE IN BLOOD

The Hoppe-Seyler<sup>9</sup> or alkali test of the blood is commonly employed in cases of carbon monoxide poisoning. The test will give a positive result if the blood is more than 15 per cent saturated with carbon monoxide. Thus, the addition of a few drops of a 10 per cent solution of sodium hydroxide to a dilute solution of normal blood immediately changes the latter's red color to the brown color of alkaline hematin. Blood containing the resistant carboxyhemoglobin in concentrations above 15 per cent when treated the same way retains its cherry red color for a considerable time. As already shown, similar results are obtained with normal blood from a human fetus or a newborn human infant having no trace of carbon monoxide in it.

Confusing results were obtained by the use of the sodium hydroxide test in an interesting case of carbon monoxide poisoning in which an autopsy was made in the Office of the Chief Medical Examiner (no. 3607-40).

A 37 year old white woman who was nine months pregnant committed suicide by inhaling illuminating gas. She was found dead in the gas-filled kitchen of her home. The body presented the typical cherry red postmortem lividity which one encounters when there is a high saturation of the blood with carbon monoxide, and the maternal blood and organs all showed the same bright red color. The uterus contained a well developed full term fetus, whose lividity was of a deep bluish color and whose blood and organs were dark colored, in contrast to the cherry red color in the mother. The fetal lungs were dark blue, firm, airless and sank in water.

Qualitative tests for carboxyhemoglobin made on the maternal blood were strongly positive, and there was no reduction in the carboxyhemoglobin spectrum after the addition of ammonium sulfide. All the chemical tests, tannic acid, sodium hydroxide, formaldehyde solution, acetic acid and ammonium sulfide, were positive for carbon monoxide. The saturation of the maternal blood with carbon monoxide was 65 per cent as determined by the Van Slyke method.

The alkali test on the fetal blood was also strongly positive, but the absence of carboxyhemoglobin in the fetal blood was indicated by the gross autopsy findings and also by all other tests for carbon monoxide, which were negative. The Hoppe-Seyler alkali test, which was the first used in this case and which is the one most commonly used in cases of suspected carbon monoxide poisoning, gave a false positive result. The fetus did not die of carbon monoxide asphyxia, as did the mother, but from simple asphyxia resulting from a lack of oxygen, which

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9. Hoppe-Seyler, F.: *Virchows Arch. f. path. Anat.* **13**:104, 1858.

is normally supplied by the maternal blood through the placenta. This case offers additional evidence that in a woman with child there is no intermingling of the maternal and fetal circulations.

Maresch<sup>10</sup> reported a somewhat similar case, in which a pregnant young woman was acutely asphyxiated by carbon monoxide after she had inhaled illuminating gas in an attempt at suicide. She was found unconscious but was resuscitated. Twelve days later she delivered a full term infant, which lived nine days and which during life presented signs suggesting a cerebral injury sustained at birth. At necropsy, in addition to pneumonia, extensive softening of the basal ganglions was found. The lesion resembled the bilateral symmetric softening of the globus pallidus that occurs in most cases of delayed death from acute carbon monoxide poisoning and led Maresch to conclude that carbon monoxide had passed through the placenta into the fetal circulation. This conclusion, however, was not altogether warranted in the case, since chemical studies of the infant's blood obviously could not be carried out before birth, at the time that the mother was asphyxiated, and since the lesion may well have resulted from simple anoxia of the fetal brain tissues and not from any specific action of carbon monoxide. Bilateral symmetric softening of the globus pallidus may result from causes other than acute carbon monoxide poisoning. There are 2 cases recorded in the literature<sup>11</sup> in which the lesion developed after acute barbiturate poisoning, and a third instance of barbiturate poisoning with a similar lesion of the brain was recently observed by one of us (M. H.) but not reported. The case which we have described indicates that carbon monoxide does not pass through the placental circulation into the fetus.

The resistance of fetal hemoglobin to the denaturizing action of alkali was also observed in a case of sudden death from toxemia of pregnancy in a woman with a nine months' fetus in utero (no. 4744-42). The maternal hemoglobin was immediately changed into alkaline hematin after the addition of 1 per cent sodium hydroxide; the fetal blood was not denaturized even many hours after the addition of the alkali.

The alkali test should not be used for the detection of carbon monoxide in the blood of newborn or very young infants, as such blood will always give a false positive result because of the strong alkali-resistant quality of its hemoglobin.

Many observations which we have made indicate that the hemoglobin of human fetuses and newborn infants is even more resistant to the denaturizing action of alkali than is the carboxyhemoglobin found in

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10. Maresch, R.: *Wien. med. Wchnschr.* **79**:454, 1929.

11. Gonzales, T. A.; Vance, M., and Helpern, M.: *Legal Medicine and Toxicology*, New York, D. Appleton-Century Company, Inc., 1940, p. 558. DeGroat A.: *Arch. Path.* **29**:271, 1940.

the blood of adults in acute carbon monoxide poisoning. In the case first described, the denaturation time of the fetal blood after treatment with 1 per cent sodium hydroxide solution was longer than that of the maternal blood, which was 65 per cent saturated with carbon monoxide.

In cases in which infanticide is suspected and in which the body of the infant has been partially incinerated, a false positive alkali test for carbon monoxide in the blood might lead to the erroneous conclusion that the infant had been burned alive. Such a case, in which the cause of death of a liveborn infant could not be determined and in which incineration occurred after death, has come to our attention.

All the other tests for carbon monoxide when used on fetal and infant blood are satisfactory and reliable. Fetal oxyhemoglobin is reduced to hemoglobin as rapidly as adult oxyhemoglobin after treatment with ammonium sulfide. Tannic acid, acetic acid and ammonium sulfide and formaldehyde solution gave reactions with normal fetal hemoglobin similar to those obtained with adult hemoglobin. In these tests grayish brown discolorations or precipitates are produced in contrast to the red color or red-colored precipitates obtained when these tests are carried out on blood containing carboxyhemoglobin. The difference in the resistance of fetal hemoglobin and adult hemoglobin to acids is insignificant and does not interfere with any of the tests for carboxyhemoglobin in which acids are used. Bischoff using twenty-five hundredth normal acetic acid found that a 1 per cent solution of human fetal hemoglobin is changed into acid hematin in twenty-three minutes, compared with adult hemoglobin, which is changed in thirteen minutes. With stronger acids, such as are used in testing for carboxyhemoglobin, there is no appreciable difference between adult and fetal hemoglobin in time taken for the formation of acid hematin.

#### DIFFERENTIATION OF HUMAN ADULT AND FETAL BLOOD AND BLOOD STAINS

The differentiation between fetal and adult blood by means of the alkali test can be carried out also on samples of blood taken during life, as well as at the autopsy table. Advanced decomposition and hemolysis of the blood do not interfere with this test. It was carried out successfully on samples of blood removed from cadavers and kept for several weeks in test tubes at room temperature. If hemoglobin is present in the decomposed blood samples, the difference in the denaturation time between fetal and adult hemoglobin is easily observed. Hemoglobin of human fetuses and newborn infants retains its resistance to alkali even when decomposed. The results of the denaturation test with alkali were in no way influenced by the cause of death.

Stains of blood from a fetus, a newborn infant and an adult were prepared on glass, paper, steel and other materials. These stains were preserved for periods of days, weeks and months at room temperature. It was observed that if a stain is only a few days old, it is not difficult to prepare an aqueous or a saline extract and that it is also possible to obtain extracts from stains which are several weeks old. The extracts from fresh blood stains can be diluted and treated with sodium hydroxide the same as fresh blood, with similar results.

Difficulty is encountered in testing blood stains which are more than several weeks old. The older stains usually have lost their red color and are brown as the result of the formation of methemoglobin during the process of drying. Extracts of these old stains are brown in color, and when examined with the spectroscope they reveal the band of methemoglobin in the red part of the spectrum. There is, however, some oxyhemoglobin which has remained unchanged and which is recognizable by its two characteristic bands in the green portion of the spectrum. If the stain is derived from fetal blood, the hemoglobin retains its strong resistance to alkali even after a long period of drying. In such cases the denaturation time can be observed only with the spectroscope, because the color of the extract of an old stain is already brown.

With extracts of old stains, the oxyhemoglobin spectrum of the hemoglobin of an adult disappears rapidly, while that of the hemoglobin of a fetus or a newborn infant persists for some time. The denaturation time of hemoglobin from old stains of blood from a fetus is shorter, however, than that of hemoglobin from fresh stains. It is usually possible to detect the presence or the absence of blood of a fetus or a newborn infant in old blood stains if extracts are prepared and treated with sodium hydroxide. A denaturation time lasting more than one-half hour indicates that the blood stain in question is from a fetus or a newborn infant or an infant less than 5 months of age. A denaturation time lasting from one to five minutes indicates that the blood stain is from an adult or from an infant over 6 months of age. In all such cases the stains are first routinely tested for the presence of human blood, and, if possible, the blood group should also be determined. Control tests with extracts of stains known to represent blood of an adult and a fetus should be made in every case for comparison. Extracts made from blood stains older than several months gave uncertain results.

#### SUMMARY

The hemoglobin of human fetuses and newborn infants is much more resistant to the denaturizing action of alkali than is the hemoglobin of adults. The denaturation time for the former when treated with 1 per



cent sodium hydroxide is from one to three or more hours; for the latter, from one to five minutes.

The alkali-resistant form of hemoglobin disappears from the blood of infants at about the age of 6 months.

The marked alkali-resistance of the hemoglobin of human fetuses and very young infants interferes with the Hoppe-Seyler alkali test for the detection of carbon monoxide in blood, producing a false positive reaction. This test is useless and misleading when used on the blood of a newborn or a very young infant. The other standard tests for carbon monoxide may be used to detect carbon monoxide in such blood.

The denaturation time of normal fetal blood that has been treated with alkali is even greater than that of adult blood containing carboxyhemoglobin treated in the same way.

In a case of acute carbon monoxide poisoning of a nine months' pregnant woman found dead in a gas-filled room, carbon monoxide was not detectable in the fetal blood although the maternal blood contained 65 per cent carboxyhemoglobin.

Decomposed and dried fetal blood can be differentiated from adult blood by means of the alkali test. Extracts of old blood stains which are brown may be differentiated by spectroscopic examination before and after the addition of alkali. The human character of such stains should be determined first. Control tests should be carried out with known extracts. Very old blood stains give uncertain results.

## Notes and News

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**Awards.**—Charles B. Huggins, professor of surgery at the University of Chicago, has been awarded the prize of \$2,000 given by Charles L. Mayer and administered by the National Science Fund of the National Academy of Sciences. This prize was offered for the outstanding contribution in 1942 to the knowledge of the growth of animal cells with special reference to cancer. The award to Dr. Huggins was made for his studies of the human prostate and prostatic cancer. A second Charles L. Mayer award will be made for work in 1943. The address of the National Science Fund is 515 Madison Avenue, New York, and nominations for the 1943 prize should be sent in by Jan. 15, 1944. The advisory committee for the fund is interested primarily in fundamental studies of the factors of growth of animal cells.

The American Association of University Women has given its achievement award of \$2,500 to Florence Seibert, Phipps Institute, Philadelphia, for her research in tuberculosis.

**Appointments.**—The Finney-Howell Research Foundation has awarded fellowships for research in cancer as follows, each with an annual stipend of \$2,000:

. Rose I. Schukoff, of Petrograd, to work at the Glasgow Royal Cancer Hospital (awarded for the third year).

Emilia Vicari, of the Ohio State University, to work at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine (awarded for the third year).

Borroughs R. Hill, of Tulane University, to work at Harvard University (awarded for the second year).

Nelicia Maier, of Paris, France, to work at Yale University (new).

James A. Miller, of the University of Wisconsin, to work at the University of Wisconsin (new).

Applications for fellowships must be made before Jan. 1, 1944 on blanks furnished by the secretary, 1211 Cathedral Street, Baltimore.

## Book Reviews

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**Atlas of Ovarian Tumors.** Gemma Barzilai, M.D., New York. Preface by Fred W. Stewart, M.D., pathologist, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. Pp. 264, 8½ by 11, with 258 original illustrations, 45 in colors, on 58 plates. Price \$10. New York: Grune & Stratton, 1943.

The illustrations, all of microscopic appearances, and the mechanical features are excellent. The legends describe well the distinctive structural patterns of the tumors; the staining method and the magnification are given in each case. In the text are good systematic accounts of the gross and the microscopic features of the tumors from the point of view of morphology; of the derivation of the tumors; of the nomenclature; of the functional activities, the course and other matters. There is, however, no systematic review of the literature. The lists of the names by which some of these tumors have been known remind the reader of their history. The author's classification is genetic, i. e., based on the cellular derivations of the tumors, and does not take into consideration their cancerous or noncancerous type. Accordingly the primary tumors are grouped as follows:

1. Derived from the follicles—granulosa cell tumor, theca cell tumor.
2. Derived from the male gonad—arrhenoblastoma, virilizing lipid cell tumor (exact nature not agreed on).
3. Derived from the early mesenchymal core of the ovary—dysgerminoma.
4. Teratoma, embryonal and adult.
5. Fibroma, sarcoma.
6. Derived from structures adjacent to the ovary or, in embryonal stages, closely entwined with the ovarian anlage—Brenner tumor, endosalpingioma, seroanaplastic carcinoma, pseudomucinous adenoma, ovarian and peritoneal pseudomyxoma, pseudomucinous adenocarcinoma, mesonephroma (Schiller).

Endosalpingioma, in group 6, is an ovarian tumor "that duplicates morphologic and functional conditions proper to the epithelium of the normal human tube." Previously this tumor has been called by many names, mostly variations of "cystadenoma" or "adenocystoma." The adjective "seroanaplastic" is applied to ovarian carcinoma in which the epithelial cells do not produce mucus, but why "anaplastic"? Are not all forms of carcinoma more or less anaplastic?

All who are interested in the newer knowledge of the derivation and the structure of ovarian tumors will welcome this atlas. It is a noteworthy accession to the literature in its field.

**Medical Jurisprudence and Toxicology.** John Glaister, M.D., D.Sc., Fellow of the Royal Faculty of Physicians and Surgeons, Glasgow; barrister-at-law of the Inner Temple, regius professor of forensic medicine, University of Glasgow; formerly professor of forensic medicine, University of Egypt, Cairo, and medicolegal consultant to the Egyptian government. Seventh edition. Pp. 679, with 132 illustrations. Price \$8. Baltimore: The Williams & Wilkins Company, 1942.

Once more this book, first published in 1902, has been revised and brought up to date. It is a textbook along traditional lines for students and practitioners, particularly those in England and Scotland. The relevant legal enactments and procedures in those countries are described in necessary detail. The part on toxicology deals with the actions of all kinds of poison, the evidence and the qualitative tests for poisoning and the treatment of poisoning, but the quantitative estimation

of poison is not considered because that is the province of the analyst. The part devoted to medical jurisprudence deals with the following topics: identification; medicolegal aspects of death; death from asphyxia (drowning, suffocation, etc.); death from electricity, lightning and burning; death from criminal neglect and starvation; the medicolegal aspects of wounds; blood stains; sexual medicolegal problems (three chapters); states of insensibility; the medicolegal aspects of lunacy. There are numerous gross illustrations, many of them highly realistic: fingerprints; dead bodies showing putrefactive and other conditions; wounds and injuries of various kinds. The illustrations in color showing a "pugilistic attitude" of a burned human body and poisonous berries and seeds are excellent. Brief instructive accounts are given of illustrative cases. The chapter on blood stains is quite satisfactory. The value of bacteriologic methods in postmortem examinations is not emphasized. The style is direct and effective. The medical student and the practitioner will find this a good practical book in its field.

## Books Received

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**MANUAL OF VETERINARY BACTERIOLOGY.** Raymond A. Kelser, D.V.M., Ph.D., brigadier general, United States Army; chief, Veterinary Division, Surgeon General's Office, War Department, Washington, D. C. Harry W. Schoening, V.M.D., chief, Pathological Division, Bureau of Animal Industry, and assistant chief, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C. Fourth edition. Pp. 719, with 94 figures. Price \$6.50. Baltimore: The Williams & Wilkins Company, 1943.

The fourth edition has been revised by Harry W. Schoening, of the United States Department of Agriculture, who has become co-author. The book is more than a manual of veterinary bacteriology because it includes also fungi, protozoa and viruses that cause veterinary infections. The part on pathogenic ultramicroscopic viruses has been rewritten and enlarged to include the recent advances in that important field of veterinary microbiology. The book continues to merit the recommendation in the review of the second edition (*ARCH. PATH.* 16:451, 1933) "as a reliable and comprehensive source of information in regard to the micro-organisms of veterinary infections."

**VASCULAR SPASM EXPERIMENTAL STUDIES.** Alexander John Nedzel, M.D., M.S., associate professor of pathology. Contribution from the Department of Pathology, Bacteriology and Public Health in the University of Illinois College of Medicine. Pp. 151, with 161 figures. Price \$2.75 cloth bound and \$2.25 paper bound. Urbana, Ill.: University of Illinois Press, 1943.

The monograph presents results of the study of the role of vascular spasm in the genesis of various diseases. Besides reviews of literature, it contains summaries of the author's previous articles and of new experiments. The action of pitressin, which was used for the production of spasm, is discussed; also the role of vascular spasm in the initiation of changes in the valves of the heart leading to endocarditis. The production by pitressin of gastric ulcers and of hepatic and renal lesions is considered. The alterations produced in the central nervous system are interpreted as analogous to those of multiple sclerosis in man.

**THE INNER EAR, INCLUDING OTONEUROLOGY, OTOSURGERY AND PROBLEMS IN MODERN WARFARE.** Joseph Fiseher, M.D., staff member, Beth Israel Hospital, Boston; formerly associate of Adam Politzer and senior otolaryngologist with Gustav Alexander of the Policlinic of Vienna. Louis E. Wolfson, M.D., instructor in ear, nose and throat, Tufts Medical School; senior surgeon in ear, nose and throat, Boston Dispensary. Pp. xii and 421, with 76 figures. Price \$5.75. New York: Grune & Stratton, 1943.

The contents are: clinical anatomy; general physiology; applied physiology; functional tests; primary diseases of the labyrinthine capsule: otosclerosis; inflammatory diseases of the inner ear; intracranial labyrinthogenic complications; chemotherapy; facial palsy; congenital diseases; neoplasms; vascular lesions: Ménière's syndrome; war trauma; the role of the inner ear in aeronautics; effects of atmospheric pressure changes on the ear.

**BRUCELLOSIS IN MAN AND ANIMALS. I.** Forest Huddleson, D.V.M., M.S., Ph.D., research professor in bacteriology, Michigan State College. Contributing authors: A. V. Hardy, M.D., Dr.P.H., associate professor of epidemiology, DeLamar Institute of Public Health, Columbia University Medical School; consultant, United States Public Health Service. J. E. Debono, M.D., M.R.C.P., professor of pharmacology and therapeutics, Royal University of Malta. Ward Giltner, D.V.M., M.S., Dr. P. H., dean of veterinary division and professor of bacteriology, Michigan State College. Revised edition. Pp. xv and 379, with 42 figures. Price \$3.50. New York: The Commonwealth Fund, 1943.

The contents are: the genus *Brucella*; methods of isolating *Brucella*; differentiation of the species of the genus *Brucella*; brucellosis in human beings; brucellosis in animals; laboratory diagnosis of brucellosis; eradication or control of sources of *Brucella* infection.

**BLOOD GROUPS AND TRANSFUSION.** Alexander S. Wiener, M.D., serologist and bacteriologist in the Office of the Chief Medical Examiner of New York City; head of transfusion division, Jewish Hospital of Brooklyn. Pp. 438, with 69 figures. Price \$7.50. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

There are two new chapters, one dealing with the transfusion of stored blood and blood substitutes, the other with the Rh factor and its role in fetal erythroblastosis. The old chapters have been extensively revised. The book presents well the present knowledge of the theory and practice of blood grouping as well as that of transfusion.

## EFFECTS OF INTRAVENOUS INJECTIONS OF THE ETHER-INSOLUBLE FRACTION OF LIPOIDS OF BEEF BRAIN

A COMPARISON WITH THE LIPOID STORAGE DISEASES AND WITH  
THE EFFECTS OF INJECTIONS OF PHOSPHATIDES ALONE

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It has been shown that phospholipids call forth macrophages and are quickly absorbed without residual reaction when they are injected subcutaneously, while galactolipids elicit chiefly neutrophils and fibrous tissue and are poorly absorbed.<sup>1</sup> When mixed with phospholipids, however, the galactolipids are absorbed and materially modify the character of the macrophages.<sup>2</sup> On the other hand, both the phospholipid sphingomyelin<sup>3</sup> and the galactolipid kerafin<sup>4</sup> are stored in macrophages throughout the organism pathologically, in association with variable amounts of glycerophosphatides,<sup>5</sup> while the glycerophosphatides themselves have not been specifically implicated in a storage disease. Sphingomyelin and kerafin have a common radical, the sphingosine-lignoceric bond, and Epstein<sup>5</sup> suggested that pathologic storage of either lipid may be due to disturbance of the metabolism of phosphoric acid alone, of such nature that hyperactivity results in accelerated linkage of the phosphoric acid radical with the sphingosine-lignoceric one and consequent overproduction of sphingomyelin, while hypoactivity results in decreased linkage, with liberation of the sphingosine-lignoceric radical for union with galactose and consequent overproduction of kerafin. Epstein<sup>5</sup> and Kimmelstiel and Laas<sup>6</sup> expressed the belief that

From the Department of Anatomy, Vanderbilt University School of Medicine.

This material was presented before the American Physiological Society (*Am. J. Physiol.* **129**:481, 1940).

1. Tompkins, E. H.: *Bull. Johns Hopkins Hosp.* **70**:55, 1942.

2. Tompkins, E. H.: *South. M. J.* **33**:154, 1940.

3. Klenk, E.: *Ztschr. f. physiol. Chem.* **229**:151, 1934.

4. Lieb, H.: *Ztschr. f. physiol. Chem.* **140**:305, 1924.

5. Epstein, E.: *Ergebn. d. allg. Path. u. Path. Anat.* **33**:280, 1937.

6. Kimmelstiel, P., and Laas, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **93**:417, 1934.

galactolipids can enter cells only in the presence of phospholipids. In view of these concepts, therefore, and the specificity of the reactions of the subcutaneous tissues to individual lipoids, it seemed important to determine the systemic effects from intravenous injections of the same lipoids when given individually or in known admixture with other lipoids.

The effects of intravenous injections of the glycerophosphatide lecithin have been reported.<sup>7</sup> The preparation of the sphingomyelins and galactolipids in sufficient quantities for repeated intravenous administration to enough animals to form representative series offered considerable difficulty. Therefore the experimental procedure was adopted of injecting into a large series of animals the biologic mixture of both substances which is present in the ether-insoluble fraction of the lipoids of beef brain and comparing the results with those obtained by injecting similarly into a smaller series of animals the purified sphingomyelins or galactolipids extracted from the mixture. Ferraro and Jervis<sup>8</sup> recently reported the pathologic changes in rabbits following repeated intravenous injections of sphingomyelin alone. The present data represent the findings obtained from intravenous injections of the mixture of sphingomyelins and galactolipids present in beef brain. They include certain aspects not investigated by Ferraro and Jervis and, in addition, serve as a basis of comparison with the effects from injections of either group of phospholipids alone, i. e., sphingomyelin and the glycerophosphatide lecithin.

#### METHODS

The material used for these experiments was purified from a dry benzene extract of beef brains which was donated by Dr. David Klein, Wilson Laboratories, Chicago.

Two different lots of material were prepared from this. The first was employed for the mice used in these studies and for the first 2 rabbits; the second was used for the remaining rabbits (3 to 13 inclusive). The crude benzene extract was dissolved in large amounts of warm ethyl ether. The solutions were allowed to stand in tall containers at 40 C. until clear. The supernatant fluids were decanted; the sediments were redissolved in warm ether, and the procedure was repeated until the supernatant fluids were practically colorless. The final sediments were stirred in several changes of warm acetone and allowed to dry in air. They were ground to a fine powder before being weighed and were made into 1 per cent stock solutions in equal parts of chloroform and ethyl alcohol. The solutions were filtered through fine paper several times in order to remove insoluble matter which had been carried along with the ether-insoluble residues. The amount of this differed with each lot. The percentages of the filtered solutions were obviously reduced accordingly.

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7. Tompkins, E. H.: *Arch. Path.* **35**:695, 1943.

8. Ferraro, A., and Jervis, G. A.: *Arch. Path.* **30**:731, 1940.

Emulsions for injection were prepared fresh each day. The desired amounts of the stock solution were measured into beakers, evaporated over a water bath, made into 5 per cent emulsions in 5 per cent dextrose solution and sterilized for two hours in a steam sterilizer.

The injections were given at the rate of one every twenty-four hours, six days a week, in the aural veins of nonpregnant adult female rabbits. The daily doses were increased gradually from 0.01 Gm. to constant levels varying from 0.05 to 0.70 Gm. (i. e., 1 to 14 cc.). The injections were given slowly over a period of three or more minutes. Under these conditions they were well tolerated with one exception (rabbit 6), both at the time of injection and throughout the experimental course. The rabbits were always quiet for several hours after receiving an injection but exhibited signs of respiratory distress only occasionally. A more con-

TABLE 1.—*Details of the Experiments*

Rabbit	Duration of Course of Injections, Days	Dosage			Weight of Rabbit, Gm.*	Hemopoietic Organs *			
		Total Amount Given, Gm.	Maximum Daily Dose			Spleen, Gm.	Liver, Gm.	Marrow	
			Amount, Gm.	Number of Days Given				Estimated Activity	Myeloid-Erythroid Ratio
1	63	2.4	0.05	46	3,530	Firm	Normal	+++	0.9
2	63	6.5	0.20	10	3,480	Firm	Normal	+++	0.8
3	36	7.5	0.35	16	2,340	Large	Large	..	...
11	20	2.8	0.35	3	2,680	Large	Large	++	...
6	51	13.9	0.50	8	2,700	3 × normal	2 × normal	+++	...
5	55	16.2	0.50	12	3,220	2 × normal	Large	++	1.7
10	57	17.1	0.50	13	3,550	3 × normal	2 × normal	++	2.7
4	59	17.2	0.50	15	3,330	11.5	150.4	+++	1.3
8	71	23.6	0.70	3	3,480	7.2	150.5	+++	3.8
13	72	23.9	0.70	4	3,400	9.8	104.5	+++	1.2
9	73	24.9	0.70	5	2,650	11.6	87.9	+++	3.5

\* The averages of corresponding weights from 9 normal control rabbits killed in the same manner as the experimental animals are as follows: rabbit, 3,800 Gm. (2,980-4,500); spleen, 2.4 Gm. (1.13-3.72); liver, 94.6 Gm. (68-110); marrow, estimated activity ++; myeloid-erythroid ratio, 1.34 (0.8-2.4).

centrated dose, on the other hand, proved immediately fatal to a rabbit<sup>11</sup> which had apparently tolerated the lower concentration without discomfort, and a dose of standard concentration but of a size usually attained only progressively also proved immediately fatal to a rabbit which had not been habituated to the experimental material. The injections were continued over intervals of time which varied from twenty to seventy-three days. The data concerning the material injected, the dosages and the duration of the course of injections are presented in table 1.

The rabbits were kept, fed, exercised and clipped exactly in the manner described for the rabbits given lecithin.<sup>7</sup> They remained in excellent condition, held or gained weight and were normally active. The fur and corneas remained healthy.

Total white and differential (supravital technic) blood cell counts were made several times a week throughout the experimental course. The blood was always drawn by puncture of an aural vein shortly before the time for an injection. Trenner automatic pipets and Levy-Hausser counting chambers were used. The



technic employed was that described for the rabbits given lecithin.<sup>7</sup> Total red cell counts were made infrequently. All statements of counts are based on absolute numbers.

Serial counts were also made throughout twenty-four hour periods following individual injections of the series. They revealed characteristic curves and presented problems which are essentially distinct from the general systemic effects. These were investigated in further detail therefore and have been presented in abstract<sup>9a</sup>; they are to be reported separately.<sup>9b</sup>

The animals were put to death by being given illuminating gas or ether or by intravenous injection of soluble pentobarbital or air in lethal amounts. As soon as respiration ceased, the abdomen was opened, the inferior vena cava cut and the blood allowed to drain, with the animal held upright. The organs were then examined grossly, and blocks were saved in Helly's or Bouin's fixatives. Blocks of the marrow were saved in Kingsley's fixative.<sup>10</sup> Scrapings of the hemopoietic organs were examined supravitaly. All tissues were routinely embedded in paraffin and cut at 3 microns, and the sections were stained with hematoxylin and eosin. The sections of marrow and those of spleen fixed in Helly's fluid were also stained with Kingsley's stain.<sup>11</sup> The sections of marrow were counted differentially. The prussian blue test for iron (Key's technic) was applied to sections of the hemopoietic organs both before and after treatment with nitric acid or trypsin for masked iron.

Twelve mice were also given injections of the lot of material used for rabbits 1 and 2. One to 5 per cent emulsions were given every two to three days in a vein of the tail, in amounts of 0.005 to 0.010 Gm. The experimental periods extended from four to sixty-four days. The tissues were studied only after fixation in Bouin's fluid.

#### EXPERIMENTAL DATA

*Studies of Blood.*—Figures 1 and 2 (rabbits 4 and 13) illustrate the reactions of the white blood cells in general. The total white cell counts began to increase ten to twenty-three days after the start of the injections (daily doses, 0.05 to 0.35 Gm.). They varied considerably from day to day but were continuously elevated throughout the remainder of the experimental period. In all instances the increase was due to an increment of neutrophils (i. e., pseudoeosinophils) and lymphocytes and in many instances to an increment of monocytes and basophils. The neutrophils began to increase sixteen to forty-six days after the start of the injections (daily doses, 0.15 to 0.50 Gm.); the lymphocytes began to increase five to forty-two days after the start of the injections (daily doses, 0.15 to 0.45 Gm.). The increase varied considerably from animal to animal and was irrespective of dosage. In some instances the counts attained levels that were double or treble the control counts. The monocytes increased in 5 animals, beginning sixteen to fifty days after the start of the injections (daily doses, 0.05 to 0.50 Gm.); they exhibited little change in the other 6 animals. The characteristic macrophages which will be shown to have infiltrated most tissues were never observed in the blood smears.

As has been stated, serial counts over twenty-four hour periods following individual injections revealed characteristic curves,<sup>9a</sup> which were found to be superimposed on the basic levels which the blood had attained at the time of the

9. Tompkins, E. H.: (a) *Anat. Rec.* **79**:59, 1941; (b) *Bull. Johns Hopkins Hosp.*, to be published.

10. Kingsley, D. M.: *Folia haemat.* **57**:87, 1937.

11. Kingsley, D. M.: *Stain Technol.* **10**:127, 1935.

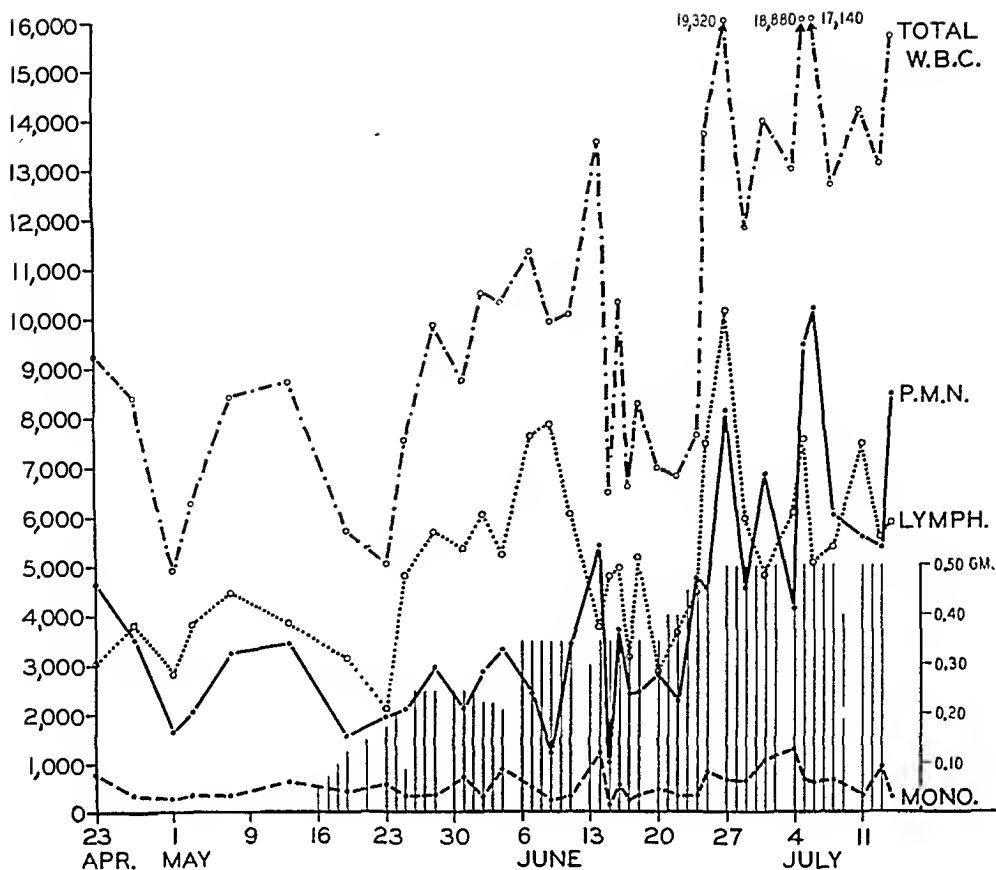


Fig. 1 (rabbit 4).—Curves of total and differential (absolute numbers) white blood cell counts correlated with the daily injections of experimental material.

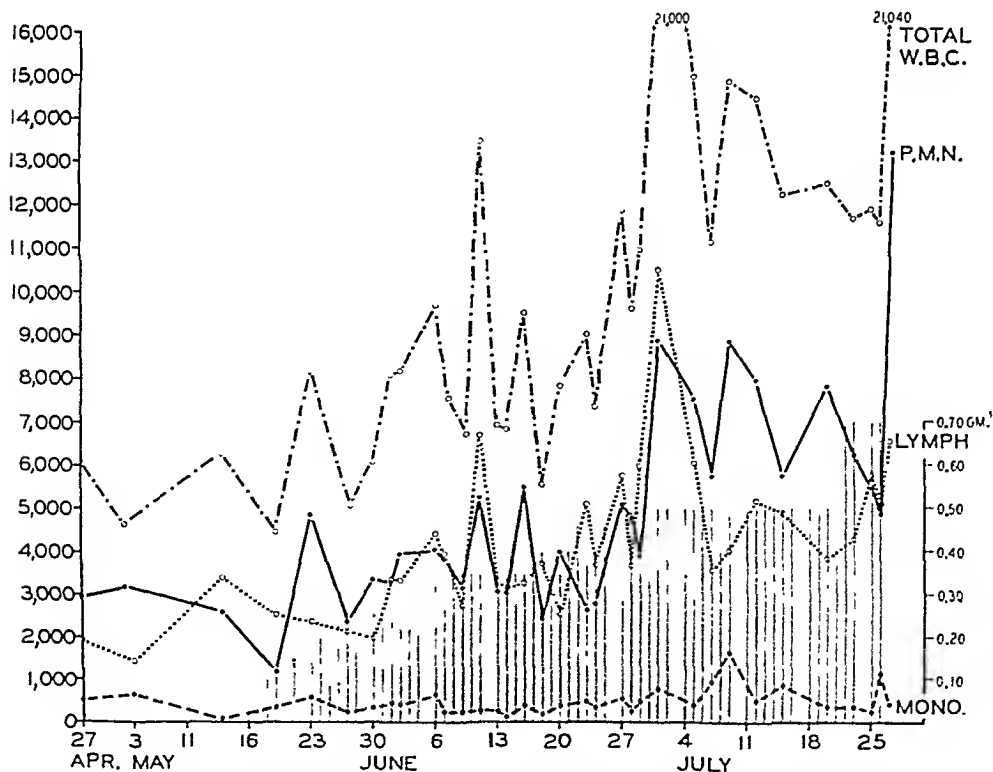


Fig. 2 (rabbit 13).—Curves of total and differential (absolute numbers) white blood cell counts correlated with the daily injections of experimental material.

particular injection. Undoubtedly, these periodic fluctuations incident to individual injections profoundly influenced the sustained changes in the white blood cell counts described. This fact, however, does not invalidate the statement that the experimental injections produced definite sustained effects on the white blood cell counts, which were characterized by increases of the total white cell counts in association with increases of neutrophils and lymphocytes invariably and of basophils and monocytes less regularly.

Red cell counts were made at approximately thirty and sixty days after the start of the injections and are presented in table 2 as of those intervals of time. With the exception of rabbit 1, which received the smallest daily dose, the counts at the end of two months were all moderately subnormal for rabbits. Nucleated red cells were observed in circulation occasionally, but qualitative changes in the erythrocytes were not encountered.

*Studies of Marrow.*—The femoral marrow was remarkable for its volume and firmness. It was deep rose in color and so firm that it could easily be handled

TABLE 2.—Red Blood Cell Counts

Rabbit	30 Days from Start of Injections		60 Days from Start of Injections	
	Daily Dose at Time of Count, Gm.	Red Cell Count	Daily Dose at Time of Count, Gm.	Red Cell Count
1...	0.05	5,410,000	0.05	5,600,000
2...	0.05	5,350,000	0.20	4,440,000
3.	0.35	4,355,000	....	.....
11...	....	.....	....	.....
6..	0.45	5,290,000	..	.....
5...	....	.....	0.50	4,820,000
10...	0.35	5,210,000	0.50	4,420,000
1..	0.35	4,600,000	0.50	4,180,000
8..	....	.....	0.50	3,900,000
13.	....	.....	0.50	4,330,000
9	...	.....	0.50	3,887,000

without trauma. Grossly, it appeared very similar from experiment to experiment, irrespective of the dosage or the duration of the course of injections. The sections (fig. 3, 1 and 2) revealed active hemopoiesis with consequent displacement of fat and, in addition, great numbers of the characteristic macrophages to be described presently. Table 1 presents the estimated degrees of hemopoietic activity of the sections and the ratios of myeloid to erythroid cells. The hyperplasia was due to increased production of granulocytes, as is indicated by the marked increase in the ratios. It was represented by generalized diffuse hyperactivity, which extended throughout the diameter of the marrow and which seemed dependent more on the duration of the experiment and the total amount of material injected than on the size of the daily dose. The cells were mostly late myelocytes and polymorphonuclears. These findings are obviously consistent with the sustained leukocytosis and increase in neutrophils found in the blood. No reflection of the lymphocytosis was observed in the marrows.

*General Pathologic Changes.*—Pathologic data concerning the hemopoietic organs are presented in table 1. Other than the evidences of increased granulocytic activity found in the blood and the marrow, the pathologic alterations were woven largely about infiltrations of huge macrophages. These were most prominent in the spleen and the lungs but were abundant in all of the hemopoietic organs and occurred

sporadically in all of the other organs. Morphologically, these macrophages were identical in all sites except the lungs, and were the same as the macrophages which had been obtained following subcutaneous injections of mixtures of the same lipoids.<sup>12</sup>

In the supravital preparations the cells varied in diameter from about 25 to 70 microns and the vacuoles from about 2 to 6 microns. The smaller cells contained only stained vacuoles, which were uniformly the shade of neutral red dye in faintly acid solutions. The largest cells usually contained entirely unstained vacuoles of the same general size as those in the stained cells but pale gray. Cells intermediate between these extremes contained mixtures of stained and unstained vacuoles, the latter fading from the deep color of those in the smaller cells to the colorless ones in the larger cells. The vacuoles were usually round to cuboidal or hexagonal in shape, rather chalky in appearance and of low refractivity. It was only rarely that highly refractive droplets of neutral fat were present among these characteristic vacuoles. The cells were rarely observed with other contents, even in the splenic scrapings. The vacuoles stained lilac with nile blue sulfate and with methylene blue and faint brownish gray with osmic acid gas, and did not stain with janus green until cytologic death was under way. Small, fine mitochondria scattered between the vacuoles stained normally with janus green. The vacuoles were not dissolved by acetone and only partly by chloroform but were soluble in methyl and ethyl alcohols and in pyridine. They obviously contained the experimental material.

In the sections these cells had a coarsely vacuolated cytoplasm, which lent to them the foamy appearance characteristic of the cells in lipid storage diseases (fig. 3, 6; fig. 4, 9, 10 and 11). The cytoplasm of the smaller cells appeared fluffy rather than actually vacuolated (fig. 4, 10), while that of the bigger cells was riddled with large vacuolar spaces of different sizes. It was slightly basophilic, dense or cloudy, and often coarsely striated. It stained deep blue with Mallory's aniline blue mixture, and the striations became intensified (fig. 4, 14). The nuclei were usually eccentrically placed and varied from reticular in the smaller cells to stippled or homogeneous in the largest ones. Binucleated or giant cells were rarely observed.

The tests for iron (prussian blue) revealed large amounts of unmasked iron between the vacuoles but practically no masked iron. The vacuolar spaces themselves did not stain but were usually rimmed with bright blue cytoplasm. Discrete dark blue deposits occurred occasionally, but usually the cytoplasm was diffusely stained, or striated, with blue, which varied in intensity from pale to azure somewhat according to site. The macrophages in the spleens and the marrows generally stained the darkest, while those in the livers rarely contained more than traces of iron.

These cells occurred in tremendous numbers in the spleens. The spleens were enlarged in all dimensions, the size being relatively proportional to the daily dose of experimental material and the total amount given. With more extreme involvement they were exceedingly firm, rose colored and gritty on section and had unusually prominent trabeculae. The sections revealed lymphoid depletion ranging from an almost normal appearance in the spleen of rabbit 1 to practically complete obliteration of the corpuscles in the more involved spleens (fig. 3, 3). The cords were swollen and densely packed with the characteristic macrophages in almost direct proportion to the lymphoid depletion (fig. 3, 4, 5 and 6). In rabbit 1, which received the smallest dose, the reticular cells were merely hypertrophied and

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12. Tompkins (footnotes 1 and 2).

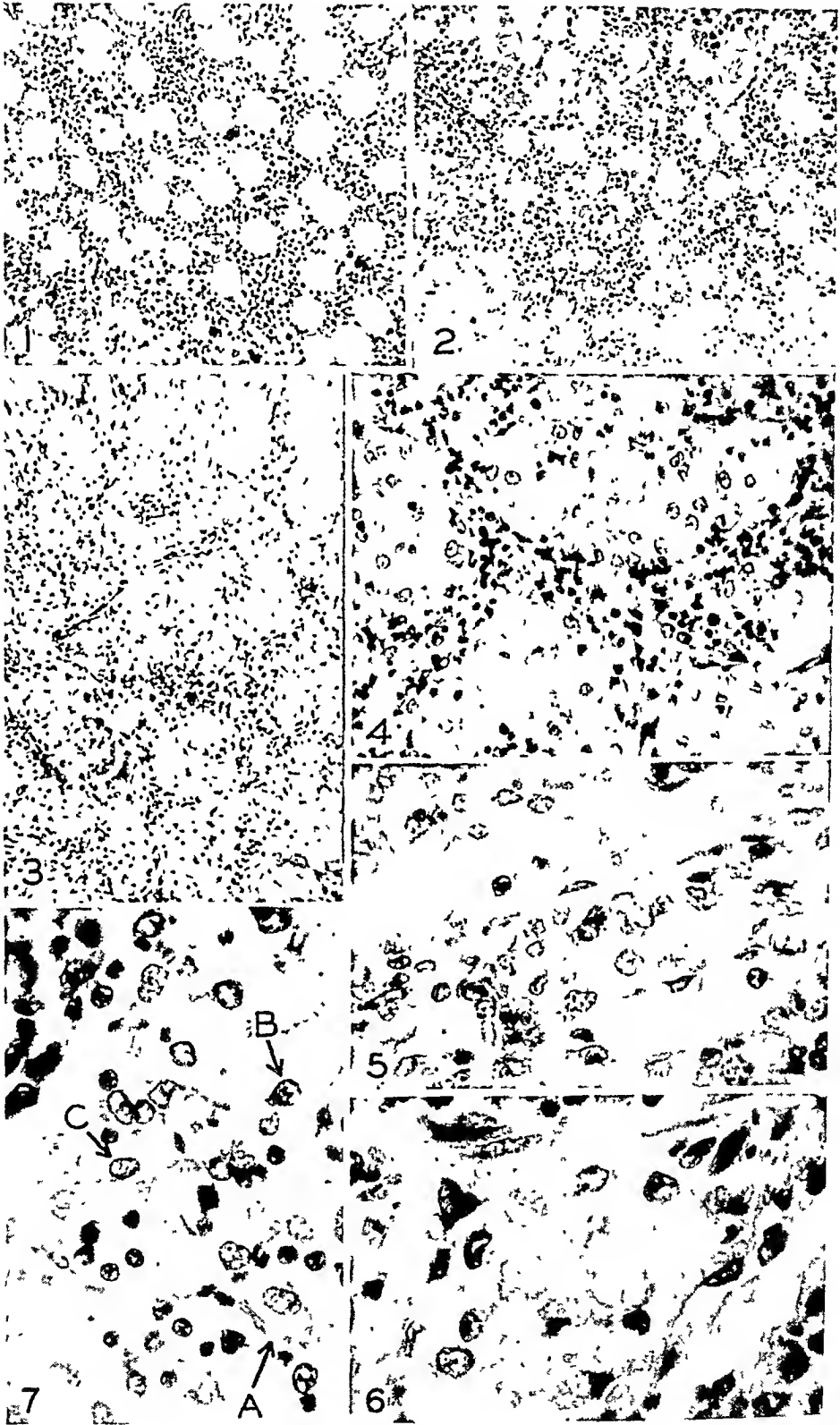


Figure 3

(See legend on opposite page)

prominent; foam cells completely replaced the reticular cells in the animals which received the larger doses (fig. 3, 4). The endothelium of the sinusoids appeared normal (fig. 3, 5 and 6). The sinusoids themselves were compressed between the swollen cords to degrees which lent a characteristic mosaic appearance to the tissue (fig. 3, 3 and 4). They contained macrophages rich in unmasked iron and similar to those of the cords, but relatively few other cells, and almost no free debris.

Macrophages also occurred in surprising numbers in the lungs. Although the organs appeared normal grossly, the supravital stained scrapings showed that they were literally swarming with macrophages, and the sections revealed definite pathologic change. In the lungs of the animals which received the largest daily doses the macrophages were similar supravital to the characteristic macrophages elsewhere. In the lungs of the animals which received smaller daily doses, however, about half of the macrophages contained small uniform vacuoles, in contrast with the large vacuoles in the macrophages elsewhere. The small size and the uniformity of the vacuoles in these cells were suggestive but not typical of epithelioid cells. Though small, the vacuoles were never extremely fine and dustlike as are those in epithelioid cells, and were rarely collected into typical rosettes. Moreover, the cells rarely contained refractive droplets of fat and were never multinucleated. This type of staining is characteristic of the cells found in supravital scrapings of any normal lung, but the numbers of such cells were far in excess of the normal. They occurred almost exclusively in the lungs of the animals given the smallest daily doses of the experimental material. On the basis of transitions, therefore, it is believed that they represent advanced stages of the characteristic macrophages found elsewhere. The sections of the lungs revealed

#### EXPLANATION OF FIGURE 3

1, marrow from rabbit 4. Central marrow from the femur, showing marked myeloid hyperplasia. Kingsley's technic;  $\times 115$ .

2, marrow from rabbit 13. Central marrow from the femur, showing marked myeloid hyperplasia. Kingsley's technic;  $\times 115$ .

3, spleen from rabbit 9. The pulp cords are crowded and distended with foam cells. The lymphoid tissue is practically obliterated, and the sinusoids are greatly compressed. A characteristic mosaic appearance results.  $\times 115$ .

4, spleen from rabbit 13. The remarks made in connection with 3 apply also here. The pale foam cells of the cords, the compressed sinusoids and the mosaic appearance are striking.  $\times 331$ .

5, spleen from rabbit 11. The remarks made in connection with 3 and 4 apply in general here. The experiment was terminated sooner than the experiments represented in 3 and 4, and the cords were not so widely distended. Foam cells are shown developing in a cord between two sinuses and are in contrast with the normal-appearing endothelial cells.  $\times 636$ .

6, spleen from rabbit 9. Higher magnification of 3. The coarse, uneven vacuolation of the foam cells and their size and character are evident in contrast with the endothelial cells. The uniformity in type of the cells in the cords is striking.  $\times 636$ .

7, mesenteric lymph node from rabbit 4. The sinusoids contain foam cells and many large nongranular and nonvacuolated cells, which are deeply basophilic. The reticular cells are enlarged and fluffy or slightly vacuolated. Arrow *A* points to a foam cell; *B*, to a basophilic cell, and *C*, to a reticular cell.  $\times 636$ .

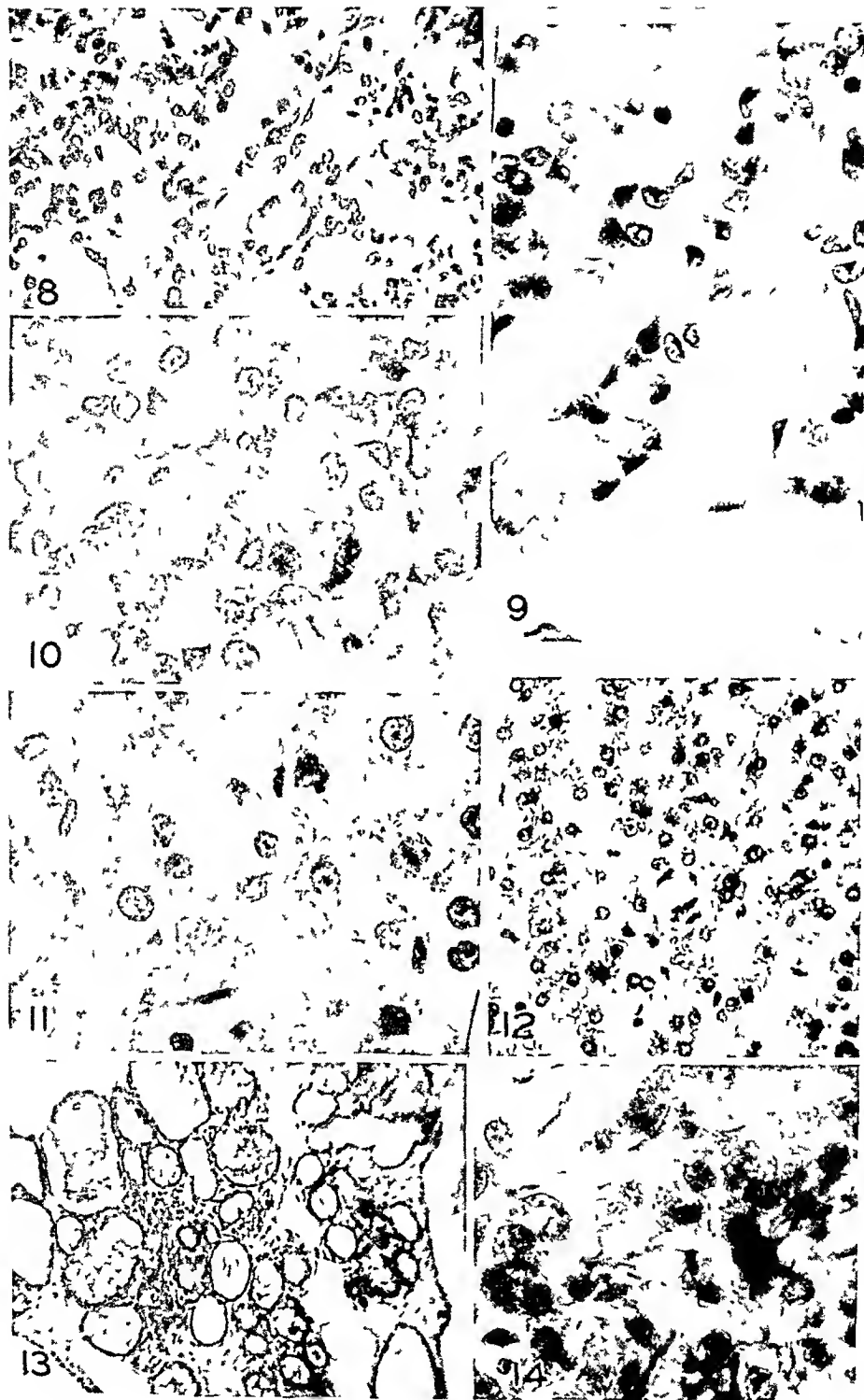


Figure 4

(See legend on opposite page)

generalized diffuse septal infiltrations and concomitant restrictions of the alveolar spaces, often to astonishing degrees (fig. 4, 8 and 10). The broad septal areas were loosely packed with cells, which varied from coarsely vacuolated ones (fig. 4, 9) to finely vacuolated, or fluffy, ones which could not be differentiated definitely from the epithelial cells (fig. 4, 10). Similar cells occurred frequently in the pulmonary alveolar spaces of the rabbits which received the largest doses, but infrequently otherwise.

Macrophages were also found in great abundance in the livers. These organs were voluminous, pale and gritty on section, and the periportal areas were prominent. In the supravital stained scrapings, clusters of the characteristic macrophages were often found surrounding the parenchymal cells. In the sections they appeared as clumps of large foam cells within the sinusoids, in numbers relatively proportional to the daily doses (fig. 4, 11 and 12). The endothelial cells about these clumps appeared normal except in the rabbits which received the largest doses, in which they were often moderately foamy. The parenchymal cells when stained supravitaly were strikingly free of fat and peculiarly homogeneous and structureless except in animals given the largest doses or in those that had shown evidence of intolerance to the injections preceding death (rabbit 6). In this respect the observations in the liver are similar to those after injections of lecithin; i. e., fat was found in the hepatic parenchymal cells only in those rabbits which received excessive doses.

The lymph nodes appeared normal grossly, but supravitaly stained scrapings revealed the characteristic macrophages in numbers somewhat proportional to the daily dose and, in addition, unusual numbers of large young cells which were nonvacuolated and contained large rod-shaped mitochondria. In sections the latter cells were found free in the sinusoids of both the cortex and the medulla; the nuclei were reticular, and the nonvacuolated cytoplasm varied from deep to pale

#### EXPLANATION OF FIGURE 4

8, lung from rabbit 13. A widespread diffuse foam cell infiltration occurred in all septums and to a lesser extent in the alveolae. As a result, the alveolar spaces are markedly constricted.  $\times 331$ .

9, lung from rabbit 4. Large clumps of foam cells distend the septums and occur infrequently in the alveolae.  $\times 636$ .

10, lung from rabbit 9. Foam cells occur in great numbers in the septums and alveolae. The alveolar spaces are constricted, and the differentiation of epithelium from foam cells is uncertain.  $\times 636$ .

11, liver from rabbit 9. Clumps of foam cells fill the sinusoids. A large clump shows in the center of the picture. These cells are in striking contrast with the slender endothelial cells, which appear entirely normal. The hepatic parenchymal cells appear normal and show strikingly little vacuolation.  $\times 636$ .

12, liver from rabbit 5. Many clumps of pale foam cells occur in the sinusoids between the cords of normal-appearing, nonvacuolated parenchymal cells.  $\times 331$ .

13, mammary gland from rabbit 10. Hyperplasia and lactation were present. The ovaries contained large follicles and generalized interstitial luteinizations, but corpora lutea were not present.  $\times 75$ .

14, subcutaneous area from a mouse given an injection of an experimental mixture of kerosin and sphingomyelin. Mallory's connective tissue stain. The cells stained deep blue, and striations of the cytoplasm are prominent.  $\times 636$ .



blue. These cells were similar morphologically and in site to cells observed in the nodes of animals given lecithin intravenously. Unlike the macrophages in other areas, those in the nodes frequently contained fine yellow granules in addition to the characteristic deposits. The reticular cells in the nodes of the animals which received the largest doses were hypertrophied and similar to the reticular cells in the spleens of the animals which received the smallest doses (fig. 3, 7).

The thymuses were voluminous, very white and somewhat firmer than normal. The reticular cells were markedly increased and moderately hypertrophied, especially those of the medulla, but frank foam cells were infrequent. They were like the reticular cells in the nodes. These changes were proportionate more to the total amount of experimental material given than to the size of the daily dose.

The adrenal glands were unusually small, often smaller than those of the guinea pig. In this they resembled the adrenal glands of rabbits given lecithin, but unlike those, they revealed no evidence of abnormality of the inner part of the zona fasciculata or of the zona reticularis. Foam cells occurred rarely in the sinusoids.

The kidneys appeared more abnormal grossly than was supported by the sections. Except in the 2 rabbits which received the smallest doses, they were pale and voluminous, and the cut surfaces had an opalescent glassy appearance which gave the impression of china or wax models. The two parts of the outer zone of the medulla merged with each other and with the cortex to an unusual degree. Supravitaly stained scrapings revealed unusual numbers of coarse red droplets in the tubular cells. The sections, however, revealed scattered foam cells between the tubules and many clumps of them within the glomerular tufts as the only abnormalities. As Ferraro and Jarvis observed after injections of sphingomyelin, foam cells were never found free in the glomerular spaces.

The mammary glands of rabbits 1, 2, 8, 9, 10 and 11 were hypertrophied and exhibited various degrees of secretory activity (fig. 4, 13). In each case, general interstitial luteinization of the ovaries was present, and the follicles were large, but there were no corpora lutea. The mammary glands of the remaining animals were inactive, the ovaries contained various-sized follicles, and the ovarian interstitial cells were slender and spindle shaped. These findings suggest that the experimental substance was capable of stimulating lactation if ovarian activity was at a stage to initiate hyperplasia of the mammary tissues, but was otherwise inactive in this respect.

The tissues from the mice given the same experimental material for variable periods were entirely comparable to those described in the rabbits.

#### COMMENT

It is obvious that intravenous injections of the ether-insoluble fraction of the lipoids from brains, containing both the sphingomyelins and the galactolipids, stimulated the production of macrophages in the same sites throughout the organism as experimental injections of the phosphatides sphingomyelin<sup>8</sup> and lecithin,<sup>7</sup> and that these sites are the same as those involved in the lipid storage diseases, namely, the reticulo-endothelial system in the marrow, the liver, the nodes and the spleen with more or less obliteration of the malpighian corpuscles and compression of the splenic sinusoids and with variable degrees of capillary accumulation of the characteristic macrophages throughout the organism.

The macrophages differed considerably from those called forth by the phosphatide lecithin in that the latter were smaller and contained iron in a masked form which was apparently so bound with lipoids that the cytoplasm was not vacuolated after treatment with fat solvents. In most respects the cells were similar to those which Ferraro and Jarvis<sup>8</sup> obtained with injections of the phospholipid sphingomyelin but differed from them in the irregularity in size of vacuoles, the frequency of striations in the cytoplasm and the abundant content of unmasked iron. In these very respects they resembled the cells characteristic of Gaucher's disease.<sup>13</sup> When stained supravitaly, they were identical with the cells called forth locally by subcutaneous injections of mixtures of sphingomyelins and galactolipids.<sup>2</sup> These were also found to resemble the cells of Gaucher's disease.

Except for these differences in character of the invading macrophages, therefore, the organic lesions in these experiments, in those of Ferraro and Jarvis with sphingomyelin, in those of Tompkins with lecithin and in the lipid storage diseases are essentially similar. The reactions of the lungs and of the hemopoietic centers represent exceptions to this generalization. Ferraro and Jarvis obtained extensive foam cell infiltrations of the lungs and marked reductions of alveolar space following injections of sphingomyelin. The pulmonary involvements in the present series of experiments were entirely comparable. In addition, periarterial accumulations of eosinophils frequently occurred. Macrophage infiltrations of the lungs following injections of lecithin, on the other hand, and restrictions of alveolar space were inconspicuous. Periarterial accumulations of eosinophils, however, were present. From the fact that the two phosphatides caused such different pulmonary changes it seems probable that the differences in pulmonary involvement under these three experimental conditions reflect merely mechanical factors concerned with differences in size and character of the invading macrophages. However, since sphingomyelin was a component of the experimental mixture used in the present series of studies as well as in the material used by Ferraro and Jarvis, it is possible that the similarities of pulmonary reaction in these 2 cases represent a specific response to that lipid.

The reactions of the hemopoietic centers in reference to the three sets of experimental injections varied markedly. The present series of injections caused sustained increases of the circulating granulocytes and lymphocytes and less regularly of the monocytes, with corresponding hyperplasia of the myeloid centers of the marrow at the late myelocytic and mature polymorphonuclear levels, but without foci of extramedullary

13. Bloom, W.: *Am. J. Path.* 1:595, 1925.

hemopoiesis. Intravenous injections of the phospholipid lecithin caused similar, though less intense, increases of the circulating lymphocytes and monocytes but, on the other hand, had little effect on the granulocytes. Since phospholipids were present in both cases, it is probable that the increases in circulating lymphocytes and also, less regularly, in monocytes represent a characteristic response to repeated intravenous administration of phospholipids. It is significant in this respect that increased activity of the lymph nodes and depletion of the splenic nodules occurred in both of these experiments and that the latter also occurred in the experiments which Ferraro and Jarvis carried out with sphingomyelin.

The marked hyperplasia of the granulocytic centers, however, which was evident in the blood and marrow in the present series of experiments was lacking in the experiments with lecithin, although extramedullary hemopoiesis occurred with formation of granulocytes as well as of erythrocytes. Ferraro and Jarvis did not report studies of the blood after injections of sphingomyelin, but they examined the marrow and noted replacement of fat by the characteristic foam cells; they made no comment as to either myeloid or erythroid hyperplasia. It is probable, therefore, that the sustained stimulation to granulocytic activity in the present experiments is related to the component of galactolipids in the experimental material. In line with this probability is the fact, which was discussed earlier, that while lecithin and sphingomyelin acted alike in stimulating local macrophage infiltrations after subcutaneous injections, similar injections of galactolipids elicited only neutrophils and fibrous tissue.<sup>1</sup> It is possible, from the method of preparation, that the biologic mixture of lipoids used in the present studies carried an impurity capable of chemotactic effect on the granulocytes. It is believed that this possibility is ruled out by the filtration of the experimental material after solution in alcohol and chloroform, by the reactions of the blood cells following single injections of the same material after treatment for removal of possible water-soluble impurities and by tests for specific impurities. These data are included in the report of the reactions of the blood cells following individual injections of the experimental material.<sup>9b</sup>

In the experiments with lecithin, in contrast with the present series, evidences of hyperactive erythropoiesis were present in the blood, marrow and spleen, and the macrophages, though filled with masked iron, contained little unmasked iron. While anemia of a similar grade developed in the present experiments and the macrophages also contained iron, evidences of increased erythropoiesis were not obtained and the iron was largely unmasked. It is unfortunate that the experiments of the present series were carried out before those with lecithin and did

not include detailed studies of the erythrocytes to serve as a more comprehensive basis of comparison in this respect. Ferraro and Jervis, likewise, made no note of increased erythropoiesis in the tissues following injections of sphingomyelin, and they failed to find iron in the macrophages. It was suggested that the anemia in the experiments with lecithin was due to the influence of that phospholipid on the resistance of erythrocytes coupled with excessive trauma within the compressed and crowded splenic sinusoids. The splenic sinusoids in the present experiments were even more compressed, yet the evidence at hand is not indicative of excessive destruction and increased production of erythrocytes, but points rather to low grades of anemia similar to those often met in conditions, including Gaucher's disease, in which there is splenic enlargement but no appreciable change in the resistance of the erythrocytes. It seems probable, therefore, that the mixture of galactolipids and sphingomyelins used in these experiments exerted little effect on the resistance of the erythrocytes and that they were able to withstand the crowded conditions in the spleen to the same degree as normal cells.

It is obvious, therefore, that intravenous injections of the biologic mixture of galactolipids and sphingomyelins used in the present series of experiments resulted in widespread infiltrations of macrophages which were like those of Gaucher's disease and in tissue changes characteristic of the lipoid storage diseases in general. In addition, they caused increases in the circulating lymphocytes and granulocytes and changes in the centers of formation of those cells which seem attributable to the phospholipid and galactolipid components of the mixture, respectively.

#### SUMMARY

Repeated intravenous injections of the ether insoluble lipids of beef brain (biologic mixture of galactolipids and the sphingomyelin group of phospholipids) cause: (a) an increase in the number of white blood cells dependent on increases in the numbers of neutrophils and lymphocytes and less regularly in that of monocytes; (b) a generalized infiltration of the reticuloendothelial organs with macrophages similar to those obtained locally after subcutaneous injections of experimental mixtures of galactolipids and phospholipids, the macrophages having the characteristics of the foam cells found in Gaucher's disease; (c) hyperplasia of the marrow with increased myelopoiesis at late levels of maturation, increase of the myeloid-erythroid ratio and infiltrations of the characteristic macrophages; (d) splenomegaly associated with massive infiltrations of the characteristic macrophages, depletion of the malpighian corpuscles

and constriction of the sinusoids; (e) diffuse pulmonary infiltrations of the characteristic macrophages with restrictions of alveolar space.

The results differ from the results of Ferraro and Jervis<sup>8</sup> with sphingomyelin by slight morphologic differences in the invading macrophages and by stimulation of the granulocytic centers. The results differ from those obtained by me with lecithin in the appearance of the invading macrophages and the presence of unmasked rather than masked iron in them, in the stimulation of the myeloid rather than the erythroid centers, in the absence of extramedullary hemopoiesis and in the presence of diffuse infiltrations of macrophages in the lungs. The organic lesions are otherwise similar to those following injections of the phosphatides alone and to those of the essential lipoid storage diseases.

# CELLULAR ORIGIN OF BRONCHIAL ADENOMA

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During the past fifteen years a number of publications have appeared describing a special group of tumors which develop in the large bronchi in youth and middle age. These are characterized by slow growth, by formation of polypoid projections which obstruct the bronchi and, in some instances, by penetration of the bronchial wall and the formation of more or less massive tumor in the surrounding lung. Metastases have been reported in the regional lymph nodes, the liver and the bone marrow (Castleman<sup>1</sup>; Adams, Steiner and Bloch<sup>2</sup>), but they are uncommon. These tumors have a distinctive morphologic appearance which has been described by many in a superficial fashion but by only a few with care and after good fixation and staining. The majority of writers have supposed that they have developed from the mucous and serous glands of the mucosa or from their ducts. This has not satisfied all observers, however, because the tumors seldom form gland spaces and it is rare indeed for their cells to secrete mucin. Womack and Graham<sup>3</sup> therefore turned to the developmental stages of the bronchi and supposed that the tumors came from undeveloped bronchial buds. They supported this hypothesis by their report of the finding of bone and cartilage in their tumors, but Tracy Mallory<sup>4</sup> pointed out that these tissues may form in pulmonary tumors as a result of metaplasia of the stroma and that the fragments of cartilage found in them may be remnants of bronchial cartilage surrounded by infiltrating tumor strands. Among the 20 specimens of bronchial adenoma studied in the Columbia University Laboratory of Surgical Pathology none shows any bone or cartilage forming an integral part of the tumor, and I agree with Mallory that the hypothesis of Womack and Graham has insufficient foundation to make it acceptable for tumors of this group. That tumors can form as the result of such developmental faults is illustrated in a case reported by Rosenblum and Klein<sup>5</sup> in which a polypoid tumor grew in the right main bronchus of an 11 year old boy and was made up of ductlike structures lined with ciliated epithelial cells and mucous glands.

The most careful and accurate histologic study of the tumors under discussion has been made by Hamperl.<sup>6</sup> He described 9 and divided them into two groups. He expressed the belief that 2 of the tumors had many features in common with the cylindromatous form of the mixed tumor of mucous and salivary glands and these he called cylindroma. He supposed that they were derived from the mucous glands of the bronchi. This is evidently a rare type. Jacob and his associates<sup>7</sup> have recorded another such tumor, and I have seen sections from a tumor of this type, although they are no longer in my possession. Clinically these tumors cannot

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1. Bronchial Adenoma, Cabot Case 26171, *New England J. Med.* **222**:721, 1940.
2. Adams, W. E.; Steiner, P. E., and Bloch, R. G.: *Surgery* **11**:503, 1942.
3. Womack, N. A., and Graham, E. A.: *Arch. Path.* **26**:165, 1938.
4. Bronchial Adenoma, Cabot Case 27511, *New England J. Med.* **225**:983, 1941.
5. Rosenblum, P., and Klein, R. I.: *J. Pediat.* **7**:791, 1935.
6. Hamperl, H.: *Virchows Arch. f. path. Anat.* **300**:46, 1937.
7. Jacob, P.; Delarue, J., and Gaultier, M.: *Bull. Assoc. franç. p. l'étude du cancer* **28**:408, 1939. Jacob, P.; Delarue, J.; Huet, P., and Depierre, R.: *Bull. et mém. Soc. méd. d. hôp. de Paris* **57**:95, 1941.

be distinguished from those of the second group, but it seems wiser to regard them as representing a different histologic type identical with the mixed tumor of salivary glands.

Hamperl's second group had the more common morphologic character, which he described in great detail. The tumor cells formed solid cords and strands, which tended to anastomose. Most of them were rounded or polygonal, and in all but 1 of his tumors, had acidophilic granules. In this seventh tumor the granules were amphoteric. Sometimes the tumor cells were elongated and resembled truncated cones. When such cells were arranged in a cord with their axes at right angles to the axis of the cord and with each alternate cell pointing in a direction opposite to its neighbor, a palisaded effect was produced. Palisaded cells sometimes outlined the larger masses of rounded cells. Occasionally the rounded cells surrounded a tiny space containing a droplet of mucoid material. The cell groups and cords frequently tended to be drawn away from the fibrous framework, leaving a free space between them. Almost all of these features have been recorded by Masson<sup>8</sup> as characterizing the carcinoid tumors of the appendix, and Hamperl was struck by the marked resemblance between the two types of tumors. There are differences, however, to which he called attention. The carcinoid of the gastrointestinal tract has granules which, after fixation in dilute solution of formaldehyde or Bouin's fluid, can be blackened with ammoniacal silver nitrate. The granules of bronchial adenoma are unaffected by the same treatment. The cells of bronchial adenoma, according to Hamperl, occasionally contain mucus which mucicarmine will redden; this is not true of the cells of gastrointestinal carcinoids. Finally, 2 of the tumors described by Hamperl contained special cell forms to which he has given the name "onkocytes." These cells are characterized by their voluminous acidophilic granular cytoplasm, which makes them differ from the other tumor cells. My observations on 20 tumors confirmed all of Hamperl's findings except in regard to the palisaded cells. I could find small ribbons of four or five cells arranged in this fashion but never any long ones. It seems proper also to make one addition to Hamperl's description. As pointed out by Zamora and Schuster<sup>9</sup> and others, and confirmed in this series, some of these tumors have an exceedingly vascular stroma and bleed freely and repeatedly. This is not a characteristic of the carcinoid tumors of the gastrointestinal tract.

Because of the resemblance to the gastrointestinal carcinoids Hamperl felt that the tumors in question should be called bronchial carcinoids, but he did not suggest for them a possible cellular origin and left this question unanswered.

It seemed remarkable to me that although Hamperl found oncocytes in 2 tumors diagnosed as bronchial adenoma, he did not investigate the bronchial mucosa to determine whether or not any of these cells could be found in it. Hamperl<sup>10</sup> is the originator of the term "onkocyte" and has expended much effort in a study of this peculiar cell type and the tumors derived from or containing oncocytes. According to him, oncocytes are epithelial cells which resemble those of the organ in which they are found but are larger and are distinguished by having in their cytoplasm distinct, markedly acidophilic granules and nuclei which either look like the nuclei of the surrounding cells or which may be more deeply stained or even appear pyknotic. He has found such cells in the salivary glands, the anterior and posterior lobes and the stalk of the hypophysis, the thyroid and parathyroid glands, the pancreas, the liver, the uterine tube and the testis. He has described

8. Masson, P.: *Ann. d'anat. path.* **1**:3, 1924.

9. Zamora, A. M., and Schuster, N.: *J. Laryng. & Otol.* **52**:337, 1937.

10. Hamperl, H.: (a) *Virchows Arch. f. path. Anat.* **282**:724, 1931; (b) *Ztschr. f. mikr.-anat. Forsch.* **27**:1, 1931; (c) *Virchows Arch. f. path. Anat.* **298**:327, 1936.

adenoma composed of oncocytes in salivary glands and has found these cells in adenolymphoma (papillary cystadenoma lymphomatosum), simple salivary gland cysts and 1 mixed tumor of salivary glands. He found oncocyte adenoma in the anterior and posterior lobes and the stalk of the hypophysis. He expressed the belief that the oxyphilic cells in adenoma of the parathyroid gland are oncocytes, and he expressed the view that the acidophilic granular cell tumor of the thyroid gland is a tumor of oncocytes. He referred to the oncocytes of the thyroid gland as Askanazy cells and was apparently unaware that in American literature they are called Hürthle cells and the tumors Hürthle cell tumors (Ewing<sup>11</sup>; Haagensen<sup>12</sup>). Finally, as already stated, he described oncocytes in bronchial "carcinoids" (i. e., adenoma).

#### PROCEDURE IN PRESENT STUDY

With the assistance of Dr. Robert C. Horn, I investigated the large bronchi of 3 mice, 2 human fetuses and 10 human adults varying in age from 19 to 80 years. All the human material was obtained at autopsies on persons who had died without primary bronchial disease except 1 adult, who had bronchiectasis. The fetuses were all aborted ones. Of the remaining 9 adults, 3 had lungs that were entirely normal, 2 had terminal edema, 1 had a pulmonary embolus, 2 had terminal pneumonia and 1 a terminal bronchitis.

The large bronchi were fixed in Bouin's fluid, and one set was immersed ninety-six hours in 10 per cent ammoniacal silver nitrate solution (Fontana's fluid, modified by Laidlaw) and counterstained with Masson's aniline blue, ponceau, acid fuchsin trichrome stain.<sup>13</sup> A second set was stained with Masson's trichrome stain without silver.

#### OBSERVATIONS

In none of the silver preparations were any cells found containing blackened granules. Of the sections prepared with Masson's trichrome stain alone, none from murine bronchi or human fetal bronchi showed cells with acidophilic granules. But all of the adult human bronchi showed scattered groups of cells with acidophilic granules both in the glands and in their ducts but not among the surface ciliated epithelial cells. These cells have all the features which Hamperl indicated were characteristic of his "oncocytes." They are the same size or somewhat larger than the neighboring cells except when the latter contain mucus; they have distinct regularly spaced cytoplasmic granules made bright red with fuchsin, and they have nuclei which either resemble those of adjacent cells or are more deeply stained and sometimes pyknotic. They occur in small groups, usually at wide intervals. Mucin has not been found in any of them. These cells are not described in the mucosa of bronchi by Heiss<sup>14</sup> or by any of the other histologists so far as I am aware.

In order to compare these cells with those composing the bronchial tumors, I photographed two acini with oncocytes in them from bronchial glands obtained at autopsies and, at exactly the same magnification, cells of three bronchial tumors (adenoma). In each case Masson's trichrome stain was used. From these photographs the composite picture illustrating this paper was compiled. It shows that the nuclear structures are comparable both in size and nucleolar arrangement. The tumor cells seem somewhat smaller and their granules not quite so regular. In color the tumor cell granules are not as deeply red. In spite of these differences the resemblance between the two is still quite striking, sufficiently so to warrant

11. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 954.

12. Haagensen, C. D.: *Am. J. Cancer* **15**:2063, 1931.

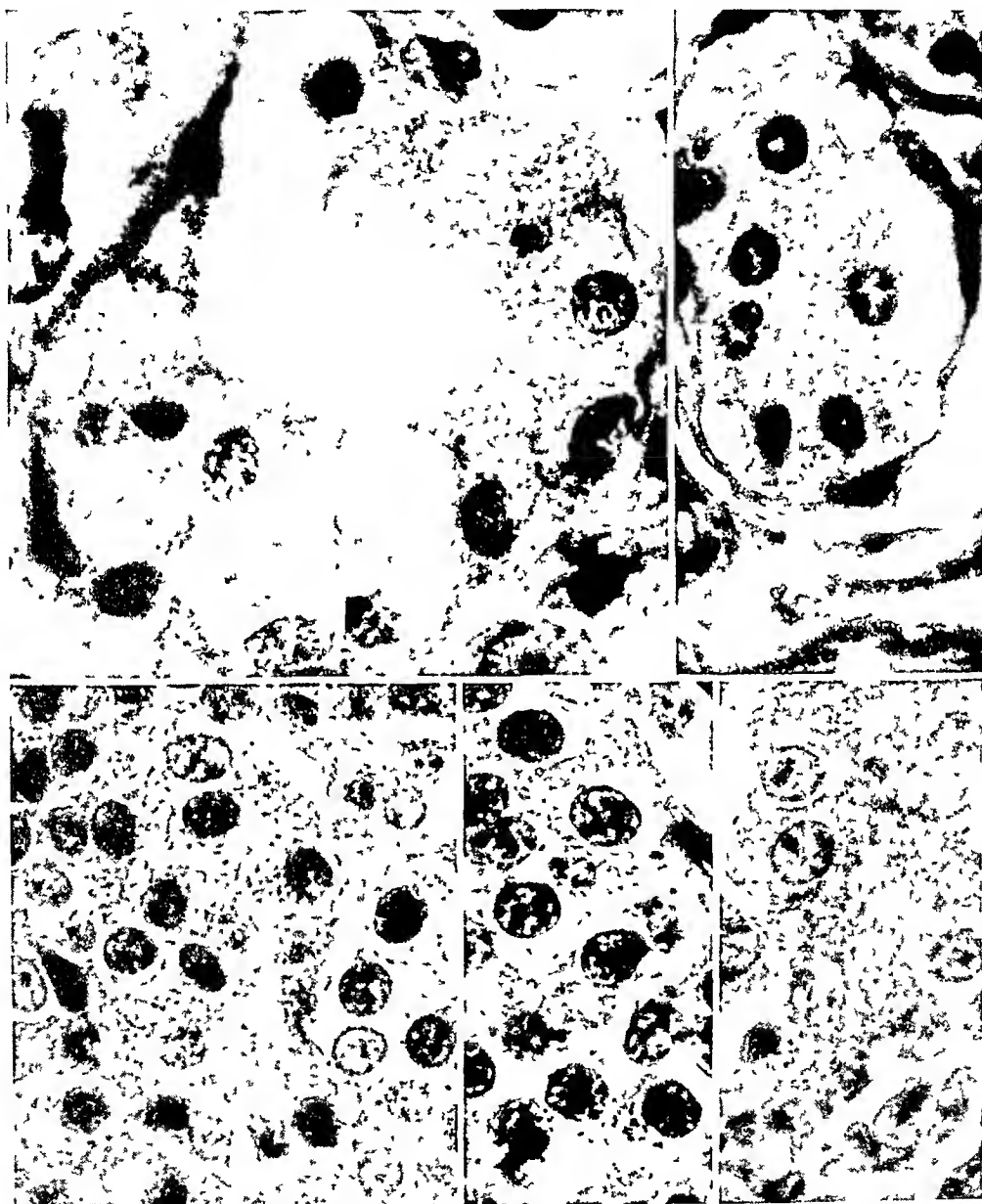
13. Masson, P.: *J. Tech. Methods* **12**:75, 1929.

14. Heiss, R.: *Der Atmungsapparat*, in von Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1936, vol. 5, pt. 3, pp. 749-750.



serious consideration of these cells of the bronchial glands with their acidophilic granules as a possible source from which the bronchial tumors are derived.

What are these oncocytes? Hamperl<sup>10a</sup> pointed out that they were first recognized in salivary glands, in 1897, by Schaffer, who called them granular swollen cells. Zimmermann<sup>15</sup> described them again, called them pyknocytes and discussed their function. He rejected Schaffer's idea that they are degeneration



The top row shows acidophilic granular cells (oncocytes, pyknocytes) in the mucous and serous glands of the main bronchi of an 80 year old woman (left) and a 37 year old man (right). The bottom row shows the tumor cells of three different specimens of bronchial adenoma. All sections were stained with Masson's trichrome stain. The composite picture is slightly reduced from a magnification of 1,330.

forms and Pischinger's supposition that they are "reserve" cells and came to the conclusion that their function is unknown. Beyond the fact that he recognized them

15 Zimmermann, K. W. Die Speicheldrüsen der Mundhöhle, in von Mollendorff, W. Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 5, pt. 1, pp. 128-129

in other parts of the body besides the primary and accessory salivary glands, Hamperl, too, has been unable to determine their functions if any. The investigations in this laboratory have established their presence in the bronchi of adults and their absence in fetal bronchi but have thrown no light on their functions.

Are the tumors diagnosed as bronchial adenoma derived from these cells? Certainly the two bronchial tumors described by Hamperl<sup>6</sup> in which oncocytes predominated must have been derived from these cells. But the large majority of the tumors grouped as bronchial adenoma are composed of cells which are not faithful reproductions of oncocytes, although they have certain resemblances to them. In spite of this, the other cells in the bronchial mucous membrane are very different indeed from the tumor cells, and one is almost forced to choose the oncocyte as the probable cell of origin, for no other cell, either in the bronchus or in the surrounding lung, offers itself as a possible candidate. The location of oncocytes in the bronchial glands and their ducts but not among the ciliated lining cells offers indirect support for this conception, since all observers are agreed that the tumors in question only secondarily involve the lining cells and could not be derived from them.

As there still remains an element of doubt about the cellular origin of this group of bronchial epithelial tumors, it does not seem proper to enter into a protracted discussion of names. There are arguments pro and con for all of the current designations. At present I use names which I believe are most widely current and therefore familiar to the largest number. I call the tumors under discussion adenoma but reject the descriptive adjective "benign" because of the aggressive infiltrative growth displayed by many of them and the rare occurrence of metastases. I recognize in addition the following: mixed tumors derived from the bronchial mucous and serous glands and similar to the mixed tumors of salivary glands, mucous gland adenoma and finally the hamartoma described by Rosenblum and Klein.<sup>5</sup> There are in addition fibroma, chondroma, lipoma, papilloma, lymphoma and a thyroid gland tumor as noted by Lindgren,<sup>16</sup> but these could not be confused with the group under discussion and are mentioned only to complete the roster of bronchial tumors other than carcinoma, sarcoma and metastases.

#### SUMMARY

The peculiar cells with acidophilic granules called oncocytes or pyknocytes have been demonstrated among the mucous and serous glands of adult human bronchi and their ducts. The relationship of these cells to the cells of bronchial adenoma is discussed, and although no conclusion is reached, it is considered possible that they may be the stem cells for tumors of this type.

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16. Lindgren, A. G. H.: *Acta oto-laryng.* **27**:183, 1939.

# BACILLUS COLI PNEUMONIA

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AND

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The problem whether *Bacillus coli* can produce pneumonia arose for us when bronchopneumonia was found at an autopsy in which the evidence pointed to this organism as the etiologic agent. The patient was a middle-aged woman with chronic glomerulonephritis who died in uremia. Material taken from the lung at autopsy, which was done almost immediately after death, yielded on culture a heavy growth of *B. coli*. Sections of lung showed large numbers of both intracellular and extracellular gram-negative bacilli. Because no other organisms except rare cocci were seen in the lesion, and because such an incitant as a virus, a toxin or a chemical could be excluded on the basis of the type of anatomic response, it seemed reasonably certain that the pneumonia was produced by *B. coli*.

The question of whether *B. coli* can cause pneumonia arose again shortly afterward when the sputum of 2 patients with pneumonia gave pure cultures of *B. coli* on repeated examinations.

A survey of the literature was of little aid in answering this question. Several cases have been presented as instances of *B. coli* pneumonia, but in most of these there was no definite proof that the pneumonia was caused by this organism, the conclusions having been based on inadequate evidence. Only one set of experiments on animals was found in which organisms believed to be *B. coli* were intratracheally inoculated. These experiments were done by Kreibich,<sup>1</sup> in 1896; he produced bronchopneumonia in rabbits, but the methods he used to identify the cultures actually did not exclude some of the other gram-negative bacilli.

In view of the paucity of information in the literature and the lack of well controlled experiments along this line, the present study was undertaken to determine whether *B. coli* can cause pneumonia when introduced into the trachea of the rabbit.

## REVIEW OF THE LITERATURE

The articles are reviewed in some detail because there is a lack of definite identification of the organisms described as "*B. coli*" and because some of the cases reported were later quoted as definite instances of *B. coli* pneumonia. In most cases detailed description of the pulmonary lesions was not recorded, but the impression was given that these lesions corresponded to those of the usual type of bronchopneumonia, consisting of a response by fibrin and polymorphonuclear leukocytes.

Sevestre<sup>2</sup> in 1887 noted that bronchopneumonia developed in many infants with infectious diarrhea. After studying many of these patients clinically and at autopsy, he decided that the bronchopneumonia was of intestinal origin. Unfortunately, he did not make bacteriologic studies. To fill this lack, an associate of his, Lesage.<sup>3</sup>

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1. Kreibich, K.: Beitr. z. klin. Med. u. Chir., 1896, no. 13, p. 1.

2. Sevestre: Bull. et mém. Soc. méd. d. hôp. de Paris 4:12, 1887.

3. Lesage: Bull. et mém. Soc. méd. d. hôp. de Paris 9:28, 1892.

studied a similar group of infants bacteriologically. In the postmortem examinations he found *B. coli* in all organs; from the lungs of those infants who also had bronchopneumonia he isolated *B. coli* in pure cultures. He expressed the belief that the *B. coli* came from the intestine. The organism that Lesage called *B. coli* was evidently a gram-negative rod. Since he did not mention sugar reactions, one cannot exclude the other gram-negative bacilli, but in all probability he was dealing with *B. coli*.

In a discussion of Lesage's paper, Sevestre<sup>3</sup> quoted Widal and Chantemesse,<sup>4</sup> who stated that in many cases of bronchopneumonia they found an organism which had all the characteristics of *B. coli*. We were unable to obtain the original article. The organism they described was most likely *B. coli*, since in an article published the previous year (1891) dealing with *B. coli* infections these authors described the use of lactose to differentiate between *B. coli* and *Bacillus typhosus*.<sup>5</sup>

Welch<sup>6</sup> stated:

. . . I have suspected that the colon bacillus may be the cause of lobular pneumonia, as in several cases this organism has been found in large number and in pure culture in congested, edematous and inflamed areas in the lungs. It has also been frequently associated with fatty degeneration of the kidneys, but neither in this nor in the pulmonary affection is there any conclusive evidence that the presence of the bacilli has done the harm.

Fischer and Levy<sup>7</sup> described 2 cases of incarcerated gangrenous hernia complicated by bronchopneumonia. In each case cultures were made of the fluid found in the hernial sac at operation, as well as of the lungs and the peritoneal exudate at autopsy. The autopsies were done ten and twelve hours, respectively, after death. In the first case pure cultures of a bacillus were obtained from all three sites, while in the second the bacillus was found together with "*Staphylococcus pyogenes albus*." They described the bacillus as a short thick rod of sluggish motility. They believed it was "*Bacterium coli commune*." No Gram stain or sugar reactions were described.

We mention a case reported by Gilbert and Girode<sup>8</sup> because their report has been quoted several times in the literature as an instance of *B. coli* pneumonia. Actually, cultures of the lung showed a mixture of *B. coli*, "*pneumococcus of Talamon*" and *Staphylococcus aureus*.

Lemoine<sup>9</sup> described a case in which bronchopneumonia caused by *B. coli* was observed as a complication of intestinal obstruction. During life he punctured the lung and obtained a few drops of bloody fluid. Smears showed a large number of bacilli and only a few diplococci. The sputum showed the same organisms, in the same proportion. Cultures of the fluid obtained from the lung by puncture showed typical colonies of *B. coli*. The bacillus was motile and fermented lactose.

Kreibich<sup>1</sup> found a case of pneumonia caused by *B. coli* in a series of 28 cases of pneumonia studied at autopsy. The autopsy in this case was done sixteen hours after death. Smears of the lungs showed gram-negative bacilli; no other organisms were seen. Material from the lungs and the bone marrow yielded pure cultures of *B. coli*. Sections of the lungs were stained with hematoxylin and eosin, with Weigert's modification of the Gram stain and with Loeffler's methylene blue. The lungs showed extensive bronchopneumonia. The alveoli were filled with exudate, which consisted mainly of serum and polymorphonuclear

4. Widal and Chantemesse: *Gaz. hebd. de méd.*, 1892, p. 16.

5. Chantemesse, Widal and Legry: *Bull. et mém. Soc. méd. d. hôp. de Paris* 8:657, 1891.

6. Welch, W. H.: *M. News* 59:669, 1891.

7. Fischer, F., and Levy, E.: *Deutsche Ztschr. f. Chir.* 32:252, 1891.

8. Gilbert, A., and Girode, J.: *Bull. et mém. Soc. méd. d. hôp. de Paris* 8:51, 1891.

9. Lemoine, G. H.: *Bull. et mém. Soc. méd. de hôp. de Paris* 11:775, 1894.

leukocytes, as well as some erythrocytes and desquamated alveolar epithelium; fibrin was sparse. In the sections stained with Loeffler's methylene blue, numerous bacilli were seen. These bacilli were seen lying free in the exudate as well as within leukocytes and desquamated alveolar epithelium. No cocci were seen either in the sections stained with methylene blue or in those stained by the Weigert method. The bacillus was gram negative. No spores were seen. The Kitasato test for production of indol was positive. There was no liquefaction of gelatin in stab cultures. The only mention made of a sugar reaction was that the bacillus fermented 2 per cent *Zuckeragar* with production of gas. We cannot be certain which sugar medium was used. Although there is no mention made of the use of lactose medium, it seems quite likely that he was dealing with *B. coli*.

Kreibich then undertook some animal experiments, using the organism isolated from this case, as well as similar bacilli obtained from 3 cases of pneumonia in which both diplococci and *B. coli* were found. After being passed through mice, the bacteria were grown on agar slants and suspended in meat broth. He injected these suspensions into rabbits in doses varying from 0.5 to 2 cc. Of 12 rabbits inoculated intratracheally, 7 showed pneumonia; of 14 inoculated intrathoracically, 11 showed pneumonia. Most of the animals died within twenty-four hours. The lesions showed a preponderance of polymorphonuclear cells and serum in the exudate, within both the bronchi and the alveoli. Some erythrocytes and desquamated alveolar epithelium were also seen in the exudate. In some cases a hemorrhagic exudate predominated. In those rabbits which lived longer (two or three days) there were also thickening and small cell infiltration of the peribronchial tissues. A rabbit that received an intravenous injection of the suspension died the next day but showed no pulmonary lesion. Cultures of the lungs were positive for *B. coli*. Sections of the lungs examined microscopically showed varying numbers of bacilli, both extracellular and intracellular. In addition, numerous shorter rods and coccoid shapes were seen, which Kreibich considered to be degenerated forms of the same bacillus. He concluded that *B. coli* commune can produce pneumonia in man.

Pearce<sup>10</sup> described 5 cases of *B. coli* bronchopneumonia in a series of 128 cases of bronchopneumonia studied at autopsy. In 2 cases the pulmonary condition was a complication of typhoid fever; in 1, of gangrene of the lung; in 1, of pulmonary thrombosis, and in 1, of acute peritonitis.

Von Schrotter and Weinberger<sup>11</sup> described a case of *B. coli* pneumonia and laryngitis which they studied clinically.

Kemp<sup>12</sup> presented a case report of a patient who, after an operation for inguinal hernia, developed "double pyelitis, cystitis, double pneumonia, purulent bronchitis, two attacks of colitis and a myocarditis . . . all due to infection with the colon bacillus." The urine showed *B. coli*. Cultures of the sputum showed enormous numbers of colon bacilli and some streptococci. The patient recovered.

Hartshorn<sup>13</sup> stated:

. . . In the respiratory tract the *Bacillus coli* is rarely the only cause of inflammatory processes. It has been found in pure culture in the sputum of patients suffering from pneumonia according to the case reports of Meara and Niles. At the Rockefeller Institute and at the Research Laboratory of the Board of Health there were no case records of pneumonia caused by the *Bacillus coli* alone.

10. Pearce, R. M.: Boston M. & S. J. **137**:561, 1897.

11. Von Schrotter, H., and Weinberger, M.: Wien. klin. Wchnschr. **21**:505, 1908.

12. Kemp, R. C.: Boston M. & S. J. **165**:819, 1911.

13. Hartshorn, M. W.: Am. J. Obst. & Gynec. **70**:482, 1914.

We were unable to find the case reports of Meara and Niles.

Felty and Keefer <sup>14</sup> described 28 cases of colon bacillus infection of the blood stream. In 5 of these there were metastatic lesions, including bronchopneumonia or septic infarction of the lung, caused by *B. coli*. These cases were proved by bacteriologic studies or by autopsy. In addition, in 4 other cases there was bronchopneumonia with bloody sputum, of which bacteriologic studies were not made, so that proof of the metastatic origin of the pulmonary disease was lacking.

Smith and Little <sup>15</sup> did some experiments on the pathogenic action of filtrates of cultures of *B. coli*. The strains were obtained from calves with a choleraform disease (scours), cultures having been made of material from the ileum. A living culture injected intravenously into a calf killed the animal in one hour; the lungs showed marked congestion and some hemorrhages. Intravenous injections of the filtrates of cultures of *B. coli* were highly toxic for calves. One calf, which was put to death two days after injection of a filtrate, showed congestion and hemorrhages of the lungs; the alveoli contained blood, fibrin and small numbers of polymorphonuclear leukocytes enmeshed in coagulum. Two other calves, which died in five and one-half and twenty-three hours, respectively, showed congestion and hemorrhages of the lungs with coagulation of blood in alveoli.

Charlton <sup>16</sup> and Helmholz and Beeler <sup>17</sup> gave numerous rabbits intravenous injections of *B. coli* and obtained various focal lesions, but in no instance did they find a pulmonary lesion.

Andreoli <sup>18</sup> presented a clinical study of the role of *B. coli* and the enterococcus in pulmonary lesions. He believed that under certain influences these organisms could become pathogenic, quit their habitat and penetrate into the circulation and that, further, they could localize in the lungs (as well as in other organs), producing pulmonary lesions of varying severity.

Ilfeld <sup>19</sup> described 3 fatal cases of *B. coli* septicemia following gastric operation. Two of the patients were examined post mortem, and both showed bronchopneumonia. No cultures of the lungs are mentioned.

Meltzer <sup>20</sup> presented the case record of a 9 day old infant who died from *B. coli* infection. The infant had bronchitis, pneumonia, meningitis and peritonitis—all caused by *B. coli*. The pus from the meninges gave a pure culture of *B. coli*. The lungs showed confluent hemorrhagic bronchopneumonia. The alveoli contained edema fluid, blood and polymorphonuclear leukocytes. There were several abscesses and necrotic areas. Meltzer also described the presence of blue amorphous bodies and meconium within the lung. Bacterial stains on sections of the lungs showed many gram-negative bacilli in bronchi, alveoli and thrombosed veins; clusters of cocci were also seen, but these were few and apparently limited to abscesses in the lungs.

#### REPORT OF A CASE

The patient was a 47 year old white woman with chronic glomerulonephritis. One month prior to death she had a severe epistaxis and, because of marked anemia, received a transfusion of 500 cc. of compatible blood. Following this, renal failure developed, accompanied by acute bilateral parotitis. Her condition worsened rapidly, and she died in uremia.

The postmortem examination was done about one and one-half hours after death. The left lung weighed 570 Gm. and the right lung 380 Gm. The bronchi contained frothy white

14. Felty, A. R., and Keefer, C. S.: *J. A. M. A.* **82**:1430 (May 3) 1924.

15. Smith, T., and Little, R. B.: *J. Exper. Med.* **46**:123, 1927.

16. Charlton, G. A.: *J. M. Research* **11**:507, 1904.

17. Helmholz, H. F., and Beeler, C.: *Am. J. Dis. Child.* **14**:5, 1917.

18. Andreoli, G.: *Arch. d. mal. de l'app. digestif* **19**:165, 1929.

19. Ilfeld, F. W.: *Arch. Surg.* **31**:632, 1935.

20. Meltzer, J.: *Geburtsh. u. Frauenh.* **1**:718, 1939.

fluid. Both lungs, especially the left, appeared edematous and congested. The lower lobe of the left lung and the middle lobe of the right lung were heavy and sank in water; they showed considerable loss of crepitation, but no areas of consolidation were made out. Material was taken from the lower lobe of the left lung and from the spleen for culture. This was done by searing the surface of the organ with a red-hot spatula and removing a wedge-shaped piece of tissue with sterile instruments. The specimen of lung yielded a very heavy growth of *B. coli* and a light growth of an alpha hemolytic streptococcus. The specimen of spleen yielded a light growth of *B. coli*. The bacillus obtained was gram negative; it fermented lactose and dextrose with production of acid and gas. Unfortunately, the cultures were discarded at this point, before the sections were examined microscopically.

Microscopic examination of the lungs showed bronchopneumonia of the lower lobe of the left lung and the middle lobe of the right lung. The alveoli were filled with an exudate consisting almost entirely of fibrin and polymorphonuclear leukocytes, although small numbers of erythrocytes and macrophages were also present. In the routine sections stained with hematoxylin and eosin, innumerable bacilli were seen lying free in the exudate as well as engulfed in the cytoplasm of macrophages. Sections of the same blocks stained with MacCallum's bacterial stain<sup>21</sup> showed these bacilli to be gram negative. Rare gram-positive cocci were also seen.

Because of the brief interval elapsing between death and the autopsy, because of the heavy growth of *B. coli* on culture of tissue from a lung and because of the tremendous numbers of gram-negative rods (present intracellularly as well as extracellularly) in the exudate, we considered that in all probability the etiologic agent was *B. coli*. The possibility of a virus, a toxin or a chemical being the etiologic agent was excluded on the basis of the type of anatomic reaction.<sup>22</sup>

The anatomic diagnosis was: chronic glomerulonephritis; slight hypertrophy of the heart; uremia; anasarca; bronchopneumonia (*B. coli*); acute parotitis, bilateral; acute ulcerative glossitis (following bites during convulsion); focal hemorrhages of the skin, the liver and the mucosa of the colon; old thrombosis of the right renal vein; thrombosis of the uterine veins; thrombosis of the left internal iliac artery; mural thrombus in the right auricular appendage of the heart.

#### EXPERIMENTAL STUDY

The purpose of the experiments was to study the effects of living and dead *B. coli*, as well as of a lysate of *B. coli*, on the lungs of rabbits.

*Materials and Methods.*—The animals used in the experiments were young adult rabbits.

The *B. coli* strains used were cultivated on Douglas agar-blood slants. Cultures of lungs were streaked on Douglas agar-blood plates. The identification of the organisms was made as follows: In each instance, smears were stained with Gram's stain, cultures were planted in lactose as well as in sucrose fermentation tubes, and the Voges-Proskauer and methyl red tests were done. These procedures were carried out with the organisms that were injected into the animals as well as with those that were recovered from the lungs of the animals at autopsy.

For the rabbits given live organisms, stock strains of *B. coli* communis and *B. coli* communior were used, as well as a strain of *B. coli* communior isolated from the sputum of 1 of the 2 patients previously mentioned. This strain was designated *B. coli* communior (P). The organisms were grown on Douglas agar-blood slants for forty-eight hours and were then suspended in sterile physiologic solution of sodium chloride. Each animal received the contents of three slants suspended in 2 cc. of the saline solution—about 50 to 100 billion organisms.

One group of animals received a similar number of heat-killed organisms. Forty-eight hour cultures of *B. coli* communior (stock) were suspended in sterile physiologic solution of sodium chloride and kept in a water bath at 63 C. for one hour. Cultures of these suspensions were negative. Each animal received the contents of three slants suspended in 2 cc. of the saline solution.

A group of animals received a toxic extract of *B. coli* communior (stock) which was prepared by repeated freezing and thawing. The contents of twelve slants (forty-eight hour

<sup>21</sup>. Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938. p. 274.

<sup>22</sup>. Sprunt, D. H.: *South. M. J.* **31**:362, 1938. Sprunt, D. H., and Camalier, W., Jr.: *Arch. Path.* **34**:801, 1942.

cultures) were suspended in 9 cc. of sterile distilled water. The suspension was frozen and thawed six times. This failed to kill all the bacteria. The suspension was then filtered through a Seitz filter, and 5.5 cc. of a clear colorless filtrate was obtained. The volume of the filtrate was made up to 8 cc. by addition of sterile saline solution. Each rabbit received 2 cc. of this filtrate.

As a control, a group of rabbits received injections of saline washings of the blood agar medium. A small amount of sterile physiologic solution of sodium chloride was washed over twelve slants, and the slants were scraped so that a blood-tinged suspension was obtained. Each rabbit of the control group received 2 cc. of this suspension.

The inoculations were made as follows: After the animal to be inoculated was anesthetized with ether, the fur of the anterior part of the neck was clipped with scissors and the skin swabbed with 50 per cent alcohol. An incision was made anteriorly in the skin and muscle of the neck, and the material was injected intratracheally with needle and syringe. The edges of the incision were then approximated with silk sutures, and iodine was poured onto the surgical wound.

Autopsies were done immediately on those animals that were put to death. Of the 3 animals that died, 2 were examined only a few hours after death and the third about fifteen hours after death. The tissues of the latter were discarded because of autolysis. Three sets of sterile instruments were used to remove each pair of lungs. The skin was covered with iodine, and was then stripped back with one set of instruments. The subcutaneous tissues were covered with iodine, and the anterior part of the thoracic cage was removed with another set of instruments. The heart and the lungs were then removed en masse with the third set of instruments. In most instances a piece of lung was cultured immediately. In some instances a piece of lung was not taken, in order to obtain better inflation of the lungs with air.

After a piece of lung was removed for culture, the lungs were inflated with air and fixed in Helly's solution. After fixation of the lungs, blocks of tissue were taken and embedded in paraffin. Sections were stained with hematoxylin and eosin and with MacCallum's bacterial strain.

*Experimental Groups.*—In all, 31 rabbits were used. All materials were injected intratracheally, each rabbit receiving a total volume of 2 cc.

Group A (rabbits 1 to 6) received living *B. coli communior* (P).

Group B (rabbits 7 to 13) received living *B. coli communior* (stock).

Group C (rabbits 14 to 19) received living *B. coli communis* (stock).

Group D (rabbits 20 to 23) received heat-killed *B. coli communior* (stock).

Group E (rabbits 24 to 27) received the lysate prepared by repeated freezing and thawing cultures of *B. coli communior* (stock).

Group F (rabbits 28 to 31) received sterile saline washings of the blood agar medium.

Except for rabbits 2, 6 and 12, which died after the injections, the rabbits were killed on the fourth day by a blow on the back of the neck. Rabbit 2 died in twenty-four hours, rabbit 6 in forty-eight hours and rabbit 12 in ninety-six hours. Rabbit 12 was discarded because of autolysis.

A summary of the experiments is presented in the accompanying table.

#### OBSERVATIONS

*Groups A, B and C.*—The lungs of the rabbits from groups A, B and C presented identical lesions and will be described together.

*Gross Changes:* All rabbits in these groups showed marked to severe degrees of pneumonia grossly. In each rabbit the great bulk of the lung tissue was involved. There were large irregular areas of consolidation, most marked in the posterior portion of the lungs, near the hilus. These appeared as dull purple areas, which were firm and airless and of rubbery consistency. In many animals there were, in addition, varying areas of hemorrhage and necrosis, brownish red in color, bounded by a pale gray border. In some lungs pronounced edema was also present. In the lungs of a few rabbits there was also a thick pleural exudate. After fixation of the lungs the cut surfaces showed similar abnormalities except that the colors differed from those of the lungs in the unfixed state. Now the dull purple consolidated regions were seen as grayish white firm areas which faded gradually into the more crepitant darker regions of the lungs. The necrotic areas, bounded sharply by a grayish white border, were now paler and stood out in marked contrast to the less inflamed regions



of the lungs, which appeared as spongy dark brown tissue. These gross appearances are illustrated in the photographs of the lungs taken after fixation (figs. 1 to 4).

**Microscopic Changes:** A pronounced degree of pneumonia was seen in all lungs. Three main types of lesions were seen. The first was an interstitial mononuclear type, characterized by marked thickening of the interstitial tissue of the lungs. This was caused by edema, congestion and marked cellular infiltration. The cellular infiltrate consisted mostly of large and small mononuclear cells, the former predominating; the former appeared to be macrophages and the latter lymphocytes. Moderate numbers of polymorphonuclear leukocytes were also present. The alveoli in many areas contained moderate numbers of large macrophages and polymorphonuclear cells, in equal proportion. Some alveoli also contained edema fluid. There was little or no fibrin. Many multinucleated giant cells were present in some alveoli and appeared to line the alveolar septums in some regions. The lining cells of the alveoli were greatly swollen and often resembled the macrophages in the lumens of the

### Summary of Experiments

Rabbit	Material Injected	Fate of Animal	Results of Culture of Tissue from Lungs		Pulmonary Lesion	Organisms Seen in Tissues
			Amount of Growth	Organism		
1	B. coli communior (P)	Killed*	++++	B. coli communior	+++	Few
2		Died, 24 hr.	++++		++++	Many
3		Killed	Culture not made		++++	Few
4		Killed	+		+++	Few
5		Killed	Culture not made		+++	Few
6		Died, 48 hr.	Discarded			
7	B. coli communior (stock)	Killed	+	B. coli communior	+++	Few
8		Killed	Culture not made		+++	Few
9		Killed	+++		+++	None
10		Killed	+++		+++	None
11		Killed	++		+++	None
12		Died, 96 hr.	Culture not made		++++	Many
13	B. coli communis (stock)	Killed	Culture not made	B. coli communis	++	None
14		Killed	Culture not made		+++	None
15		Killed	++		++++	Rare
16		Killed	+		+++	Rare
17		Killed	+		+++	None
18		Killed	Culture not made		+++	None
19	Heat-killed B. coli communior (stock)	Killed	Culture not made	.....	++	Few
20		Killed	None		++	Few
21		Killed	None		+++	Few
22		Killed	None		+++	Few
23	Lysate of B. coli communior (stock)	Killed	None	.....	++	None
24		Killed	None		+	None
25		Killed	None		+	None
26		Killed	None		++	None
27	Saline washings of medium	Killed	None	.....	None	None
28		Killed	None		None	None
29		Killed	None		None	None
30		Killed	None		None	None
31		Killed	None			

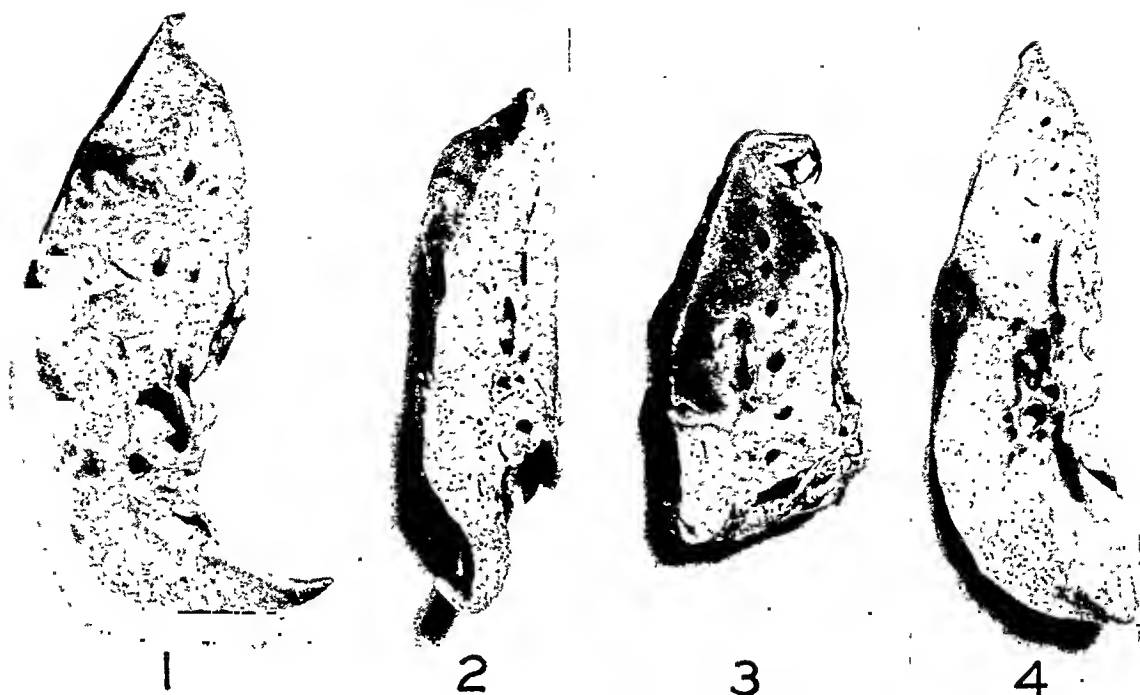
\* Killed on the fourth day.

alveoli. The bronchi were loaded with pus and often showed ulceration. The lymphatics were filled with debris, fluid and inflammatory cells. Many blood vessels showed a considerable round cell infiltration of all coats and some intimal fibroblastic proliferation. The pleura in a few cases was covered with a thick fibrinopurulent exudate. This type of lesion is illustrated in figures 5, 6 and 7.

The second type of lesion consisted of infarct-like areas of necrosis (figs. 5 and 8). These regions showed a great deal of karyorrhexis. In many areas the shadowy outlines of the alveolar septums were still visible, and here the alveoli contained large pink-staining structures, apparently swollen macrophages which had been destroyed. In these necrotic areas the blood vessels showed a purulent and necrotizing inflammation accompanied by thrombosis. The areas of necrosis may have been due to a greater concentration of bacteria in these regions.

The third type of lesion consisted of a plugging of alveoli with masses of pink-staining material, which appeared to be conglomerated erythrocytes in various stages of degeneration.

Sections of lung stained with the bacterial stain showed small numbers of gram-negative bacilli; these were seen lying free in the exudate as well as within macrophages. In addi-



Figs. 1 to 4.—Photographs of rabbit lungs fixed in Helly's fluid. The paler areas are the severely inflamed and necrotic portions. The lungs are from rabbit 5 (fig. 1) rabbit 15 (fig. 2), rabbit 3 (fig. 3) and rabbit 4 (fig. 4). The lung of rabbit 3 (fig. 3) shows necrosis and thick pleural exudate. In the lung of rabbit 4 (fig. 4) the grayish white areas are the firm rubbery consolidated parts of the lung.

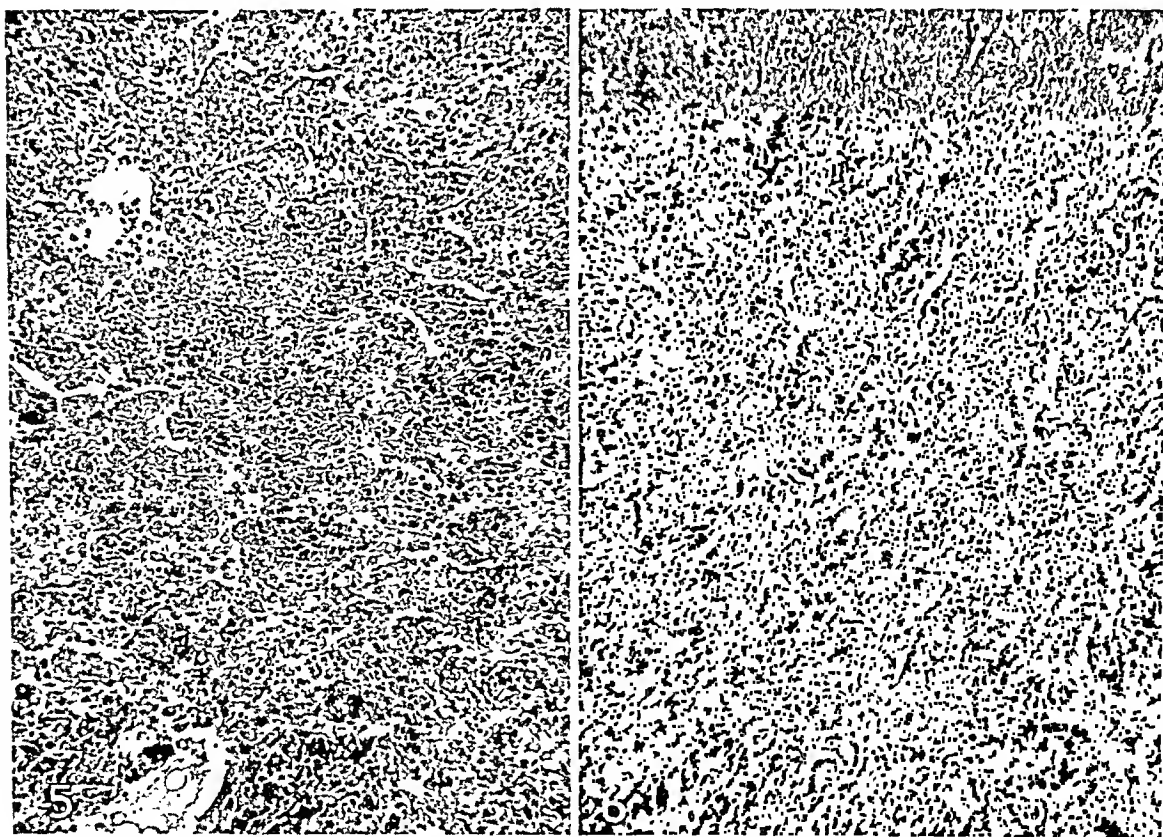


Fig. 5.—Section of an inflated lung of rabbit 20, showing the characteristic lesion. Note the necrotic area in the upper right hand corner (hematoxylin and eosin;  $\times 115$ ).

Fig. 6.—Section of an inflated lung of rabbit 27, showing marked thickening of the interstitial tissues (hematoxylin and eosin;  $\times 115$ ).

tion, large numbers of very short gram-negative rods and coccobacillary structures were seen within macrophages, presumably degenerated forms of *B. coli*. No other organisms were seen in the lungs. The only lungs which presented large numbers of the gram-negative bacilli were those of the rabbits which had died (rabbits 2 and 12). In these rabbits the greatest numbers of organisms were seen in the necrotic areas. It is possible to account for the large numbers of bacilli found in these two animals by postmortem proliferation of the organisms. On the other hand, it is just as likely that during life the bodily defenses of these rabbits were unable to prevent the rapid multiplication of the bacilli, which multiplied quickly and killed the animals; this could explain the death of the animals and the presence of numerous bacteria in the lungs.

In groups A, B and C, the lungs were cultured in half the cases; in all of these *B. coli* was recovered in pure culture. In groups A and B the organism recovered was *B. coli* communior, and in group C, *B. coli* communis. Thus, in each case the type of organism recovered corresponded to the one that was originally inoculated.

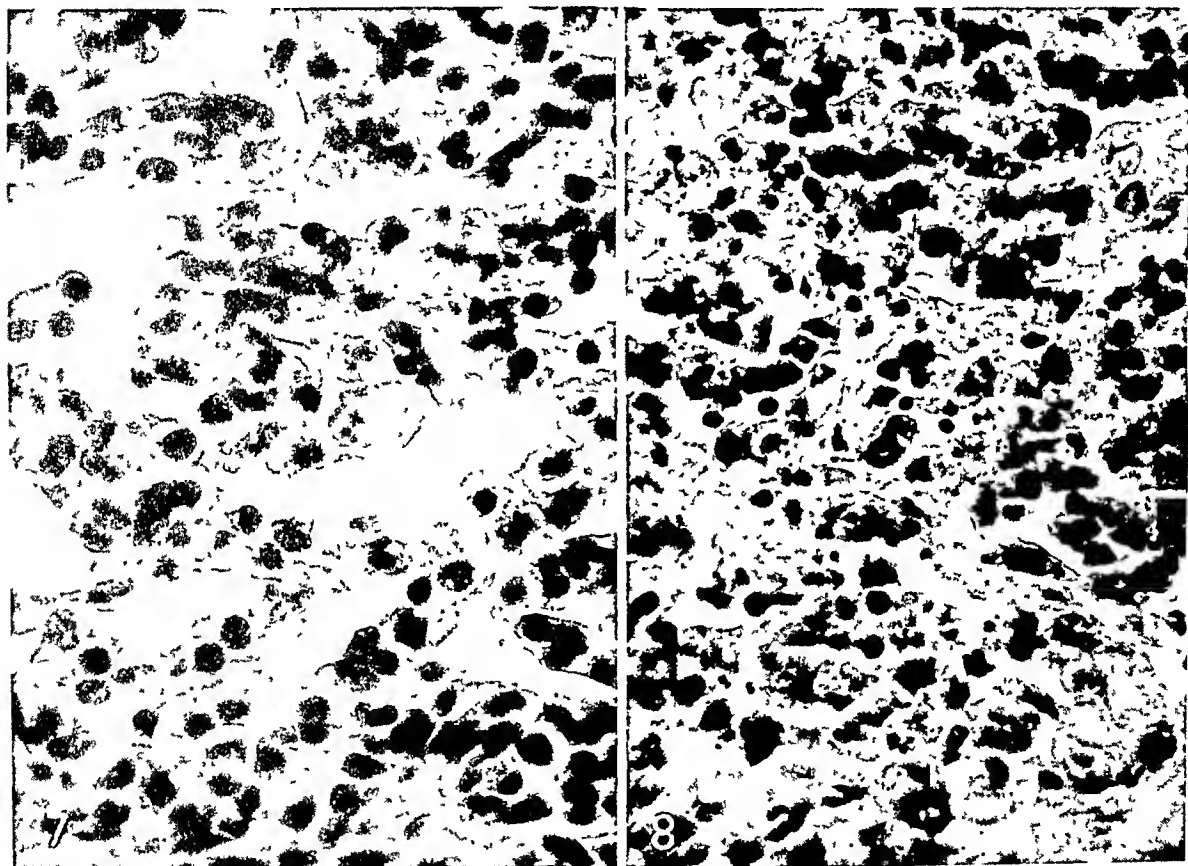


Fig. 7.—Section of an inflated lung of rabbit 7. Note the marked infiltration of the interstitial tissues by mononuclear cells, as well as the macrophages in the alveoli (hematoxylin and eosin;  $\times 687$ ).

Fig. 8.—Section of an inflated lung of rabbit 20, showing an area of necrosis. Note the karyorrhexis and the swelling of the lining cells of the alveoli (hematoxylin and eosin;  $\times 687$ ).

*Group D.*—The rabbits which received the dead organisms had essentially the same types of pulmonary lesions as the rabbits which received the live organisms except that generally the lesions were less severe. The lesions were moderate in 2 animals and moderate to marked in the other 2.

Sections stained with the bacterial stain were identical in appearance with those of groups A, B and C. Few gram-negative bacilli and numerous degenerated forms, both intracellular and extracellular, were seen. No other organisms were seen.

Cultures of tissues from the lungs of all 4 rabbits were negative.

*Group E.*—The rabbits which received the lysate also had lesions essentially similar to those in the previous groups but much less marked in degree and extent. Only a small

portion of the total pulmonary tissue was involved in each rabbit. In 2 rabbits the lesion was slight, and in the other 2, moderate. Focal necrosis was present in only 1 animal. The chief response was marked interstitial thickening, caused by edema, congestion and mononuclear cell infiltration (fig. 6).

No organisms were seen in the tissues. Cultures of material from the lungs of all 4 rabbits were negative.

*Group F.*—The lungs of the animals which received the saline washings of the medium revealed no lesions except slight congestion and slight leukostasis of the capillaries. No organisms were seen in the tissues. Cultures of material from the lungs of all 4 rabbits were negative.

#### COMMENT

After the information obtained from the experiments was put together with that from the case of pneumonia studied at autopsy, it was concluded that *B. coli* can cause pneumonia in man. The fact that pulmonary lesions were obtained with dead organisms and a lysate of the organisms as well as with live organisms seems to point to the action of a toxin of *B. coli* as one of the etiologic factors. There was a difference between the type of reaction in the human lung and that in the rabbit lung. The former showed fibrinopurulent exudation into the alveoli, while the latter showed interstitial mononuclear pneumonia with focal necrosis.

Apparently, pneumonia caused by *B. coli* is uncommon in man, but we suspect that many cases have been missed because of the general tendency to attach little or no importance to the presence of this organism in laboratory specimens, especially those obtained at autopsy. This is particularly true of specimens obtained from the lungs because, although the importance of the role of *B. coli* in infections of the genitourinary tract and the gastrointestinal system is generally appreciated, there is little information in the literature about the effect of *B. coli* on the lungs. There is no doubt that in most instances the isolation of *B. coli* from autopsy material signifies contamination or, perhaps, postmortem invasion of tissues by the organism; still, the consideration that the presence of *B. coli* in these specimens may be of some significance may result in finding more pulmonary lesions caused by this organism.

The question arises as to how the organism reaches the lungs and from what sources.

Kreibich<sup>1</sup> stated that an infection of the lungs with *B. coli* as a result of aspiration cannot be excluded, that at least it seemed probable in a case of carcinoma or of a diverticulum of the esophagus with break-through into the trachea or in a case in which vomited material was aspirated. He thought, however, that in most cases *B. coli* pneumonia was caused by hematogenous infection, either from the bowel or from inflammatory processes of the urogenital tract.

Felty and Keefer<sup>14</sup> in their discussion of *B. coli* septicemia listed the portals of entry of the organism into the blood stream in their series of 28 cases. The portal of entry was the urinary tract in 16 cases, the female genital tract in 6, the intestinal tract in 2 and a wound infection in 1; in 3 cases it was undetermined. They quoted the results of Jacob's<sup>23</sup> studies as follows:

In Jacob's compiled cases, the biliary passages were most frequently found as the primary focus; after this in order of frequency were the urinary tract, the intestine and the female genital tract. Cases of *B. coli* sepsis in which the intestine was regarded as the portal of entry either followed acute inflammatory processes of the intestine (typhoid, dysentery, etc.) or developed subsequent to appendix abscesses with thrombophlebitis or peritonitis.

Andreoli<sup>18</sup> expressed the belief that the gastrointestinal tract was often the source of pulmonary infections and that the infecting organisms reached the lungs

23. Jacob, L.: Deutsches Arch. f. klin. Med. 97:303, 1909.

through the general circulation. He quoted the experiments of Moscati and of Binet and Loubry,<sup>24</sup> who demonstrated in the dog a connection by way of lymphatics between the abdominal cavity and the lungs. Rouviere,<sup>25</sup> however, referring to these experiments, stated that the anatomic disposition which assures this manner of communication in the dog does not exist in man.

Ilfeld,<sup>19</sup> in discussing fatal cases of *B. coli* septicemia following gastric operations, stated:

. . . It seems likely that the organisms were in the stomach at the time of operation and were thus introduced into the blood stream. It is well known that the stomach may contain *B. coli*.

He then quoted several authors who had reported finding this organism in the gastric juice.

Since *B. coli* may be present in gastric juice in some instances, it seems fairly reasonable to assume that aspiration of the contents of the stomach may introduce these organisms into the lungs.

To summarize, it seems that *B. coli* can reach the lungs by being aspirated and by being carried there in the blood stream. In regard to man it seems unlikely that the organisms can reach the lungs from the gastrointestinal tract by means of direct lymphatic channels.

— In our human case of *B. coli* pneumonia, aspiration may have been the method of entry of the bacteria into the lungs. The patient was comatose, and aspiration of the contents of the nasopharynx or of the stomach under such circumstances is quite common, if not the rule. We cannot be certain of the source of the organisms, but we do know that the patient had acute ulcerative glossitis following biting of the tongue during convulsions. Sections of the tongue stained with MacCallum's bacterial stain showed many gram-positive cocci and bacilli and many gram-negative bacilli. ~~It may be that the *B. coli* pneumonia resulted from aspiration of the organisms which may have been present in the mouth.~~ Sections of the parotid gland stained with the bacterial stain showed only gram-positive cocci.

#### SUMMARY AND CONCLUSIONS

The literature on pneumonia caused by *B. coli* is scanty and, for the most part, inadequate and inconclusive. A case of pneumonia in man studied at autopsy is presented, in which it seemed reasonably certain that the etiologic agent was *B. coli*. The response was a fibrinopurulent exudate filling the alveoli and bronchi. This type of reaction excluded the possibility that a virus, a toxin or a chemical was the etiologic agent.

Intratracheal injections of live cultures of *B. coli* into rabbits produced interstitial mononuclear pneumonia and focal areas of necrosis. Similar lesions, although of lesser severity, were produced by using heat-killed cultures of *B. coli* and a lysate obtained from cultures of *B. coli*. Thus it seemed that a toxin of *B. coli* was one of the etiologic factors in the production of the lesions in the rabbits.

It is concluded that *B. coli* can produce pneumonia in man and in rabbits.

A difference is noted between the response in the human being—fibrinopurulent pneumonia—and the response in the animals—interstitial mononuclear pneumonia.

24. Binet, L., and Loubry, J.: Bull. Acad. de méd., Paris **94**:1276, 1925.

25. Rouviere, H.: Anatomie des lymphatiques de l'homme, Paris, Masson & Cie, 1932, p. 218.

# CONGENITAL ANEURYSMS OF THE CEREBRAL ARTERIES

AN EMBRYOLOGIC STUDY

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BOSTON

The occurrence of actually congenital aneurysms of the arteries at the base of the brain has often been doubted, many writers considering all aneurysms essentially pathologic. The present paper seeks to answer the question by a study of the development and growth of the arteries of this system and by a detailed examination of these vessels in subjects of various ages, from embryo to adult. In the literature there are scattered drawings of the cranial arteries, included in numerous general descriptions of particular embryos, and Mall<sup>1</sup> made a special study of the early cerebral system, but nowhere, to my knowledge, have the developmental changes been consecutively followed through prenatal life and compared critically with the conditions present at birth and in later years. For this reason, and also because these changes were found to bear so directly on the subject, it seemed necessary to include such a study here.

The growth of the brain and of the cranial arteries can be followed in the series of embryos shown in figures 1 to 5. The first two of these are reconstructions of pig embryos, since no suitable human material of these younger stages was available, but there is no reason to suppose that any essential species difference is present at these early stages. The other figures represent the conditions found in human embryos from the Harvard Embryological Collection. At 5 mm. the brain already exhibits the three primary vesicles, forebrain, midbrain and hindbrain, and is bent so that the morphologic tip, carrying the optic vesicles, points caudally. The earliest branch of the aortic arch forms the trunk of the future internal carotid artery and, growing forward, encounters the optic vesicle first, sends branches above and below it and continues along the under side of the brain, following its lesser curvature to the base of the hindbrain, where it is lost in a net of capillaries. At this age all the cerebral arteries belong to the carotid system, since there is no connection with the future vertebral vessels.

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From the Harvard University Medical School.

1. Mall, F. P.: *Am. J. Anat.* 4:1, 1904.

Distal to the vessels to the eye, numerous capillaries spring at right angles from the trunk toward the brain. The arrangement is bilateral, with no connection between the two sides.

In the embryo of 7 mm. the subdivisions of the brain are more definitely marked, especially in the forebrain, where the optic vesicle has become stalked (and is represented as cut through this narrower portion) and displaced ventrally by the growth of the hemisphere. Together these two structures occupy the tip of the forebrain or telencephalon; the rest of the forebrain is known as the diencephalon and is destined to produce the thalamus and subthalamic structures. The capillaries to the brain have lengthened and by union of branches have formed a close-meshed rectangular net, with some members running parallel to the trunk. The first branch of the arterial trunk, below the optic stalk, remains small. The second branch, arising from a network, divides further, one branch running behind the swelling of the hemisphere, one to the middle of it and one to its ventral side. They may be recognized respectively as the anterior choroid, middle cerebral and anterior cerebral arteries. The last continues on toward the nasal organ, now represented as a shallow pit of surface epithelium on the ventrolateral tip of the head. The anterior cerebral artery is thus shown to be primarily the artery of the olfactory organ, though later this connection is represented only by a minor branch. The posterior cerebral and superior cerebellar arteries are recognizable as two ill defined groups of peripheral branches arising from a nearly continuous net. As shown by Evans,<sup>2</sup> whose figures agree closely with mine, a full injection of the vessels of the head reveals that the capillary net is continued into veins lying peripheral to the arteries. All the early branches run along the side of the brain without piercing the brain substance.

The human embryo of 17.8 mm., probable age 7 weeks (fig. 3), has been fully reconstructed by Thyng<sup>3</sup> and is still available for a further detailed study of the cerebral arteries. The hemisphere extends beyond the limit of the median forebrain rostrally and overlaps it caudally. The optic stalk is relatively smaller and more ventrally placed. A former minor branch of the anterior cerebral artery has become the main trunk and extends with a sharp curve dorsally over the end wall of the median forebrain, the lamina terminalis, under cover of the overhanging hemisphere, to supply the choroid plexuses of both the third ventricle and the lateral ventricle where they meet rostrally

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2. Evans, H. M.: Development of the Vascular System, in Keibel, F., and Mall, F. P.: *Manual of Human Embryology*, Philadelphia, J. B. Lippincott Co., 1912, vol. 2, pp. 570-709.

3. Thyng, F. W.: *Am. J. Anat.* **17**:31, 1914.

above the foramen of Monro. At the ventral side of the hemisphere, where the two anterior cerebral arteries, right and left, make their sharp turns dorsally, they run close to each other and give off several sprouts, which anastomose to form an intricate plexus, the future anterior communicating artery. The middle cerebral artery has become more definite, its branches spreading over the lateral surface of the hemisphere. The numerous vessels previously recognized as a group representing the posterior cerebral artery are now in the process of becoming branches of a single trunk by the absorption of all but one of the roots of the capillary plexus from which they formerly arose. The branches cover the lateral wall of the diencephalon and also of the midbrain. One branch runs forward under the caudal pole of the hemisphere to supply the choroid plexus of the third ventricle from behind. The corresponding vessel on the opposite side sprouts directly from the main trunk. Mall called a closely similar vessel the anterior choroid artery, which it almost certainly is not, for the latter is represented more rostrally, running partly under cover of the hemisphere, arising from what can now be recognized as the future continuation of the internal carotid artery. The vessel in question is more properly a primitive posterior choroid artery, later replaced by another more peripheral branch of the posterior cerebral artery. The two lateral basilar arteries have joined with the vertebral arteries and have fused along the base of the hind-brain to a point between the roots of the superior cerebellar artery (which has also acquired a single stem) and the posterior cerebral trunk. From all these various arteries short capillary branches, usually arising at right angles, now penetrate the brain substance.

From this point the history of the cerebral arterial pattern, the first steps of which are evident in an embryo of 45 mm. (fig. 5), is governed by the further growth of the hemispheres, the development of the basal ganglions and of the ventral nuclei of the midbrain, and by the addition of the large fiber tracts. The thalamus represents the growth of many nerve cells in the dorsolateral wall of the diencephalon, causing the brain wall to thicken and bulge laterally. The caudate and lenticular nuclei develop in the floor of the hemisphere, the caudate bordering the ventricular cavity, the lenticular in a more ventral and lateral position. As the hemisphere assumes its older curved form, the head of the caudate nucleus bulges forward beyond the foramen of Monro, while the body arches backward over the lenticular nucleus. The nerve fibers comprising the internal capsule course between the two and continue downward peripheral to the thalamus, where they are originally surface fibers, even as in the peduncles, but are soon covered in by the fusion, from the foramen of Monro backward, of the lateral wall of the forebrain with the mesial wall of the overhanging hemisphere. This progressive



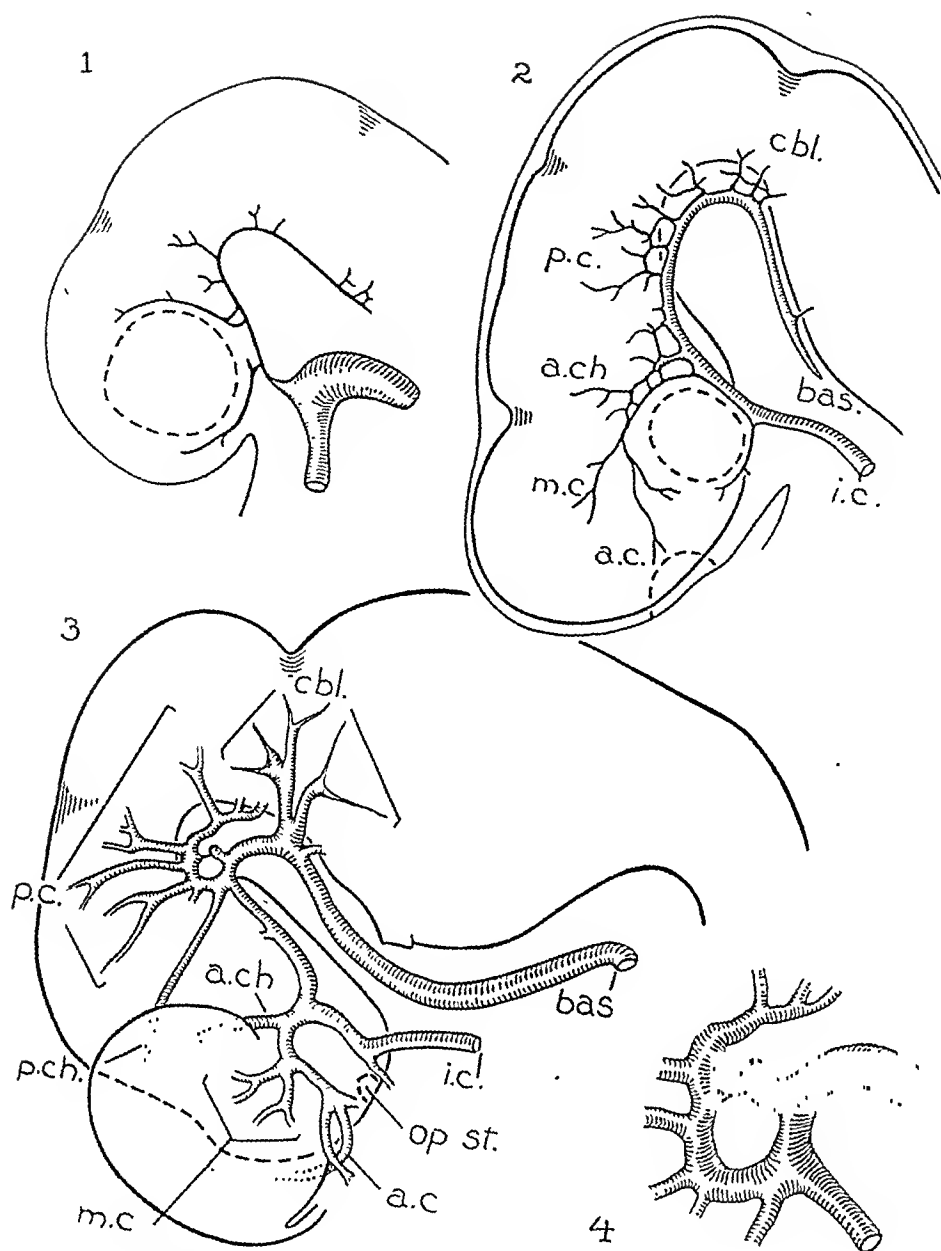


Fig. 1.—Arterial supply to the head of a pig embryo of 5 mm. The positions of the forebrain, with optic vesicle, the midbrain and the hindbrain are indicated. Note the cerebral plexus from the main trunk arising from the aortic arch.

#### ABBREVIATIONS

*a.c.*, anterior cerebral artery  
*a.ch.*, anterior choroid artery  
*bas.*, basilar artery  
*cbl.*, superior cerebellar artery  
*ch.*, optic chiasm  
*c.pl.*, commissural plate

*i.c.*, internal carotid artery  
*m.c.*, middle cerebral artery  
*op.st.*, optic stalk  
*p.c.*, posterior cerebral artery  
*p.ch.*, posterior choroid artery  
*p.com.*, posterior communicating artery

Fig. 2.—Arterial supply to the brain of a pig embryo of 7 mm. The hemisphere is appearing above the optic vesicle. The individual cerebral arteries are represented by groups of plexuses. The anterior cerebral artery runs to the nasal pit. Further explanation is given in the text.

Fig. 3.—Brain and cerebral arteries of a human embryo of 17.8 mm.

Fig. 4.—Enlargement of a portion of figure 3, showing formation of a single stem from a plexus representing the posterior cerebral artery.

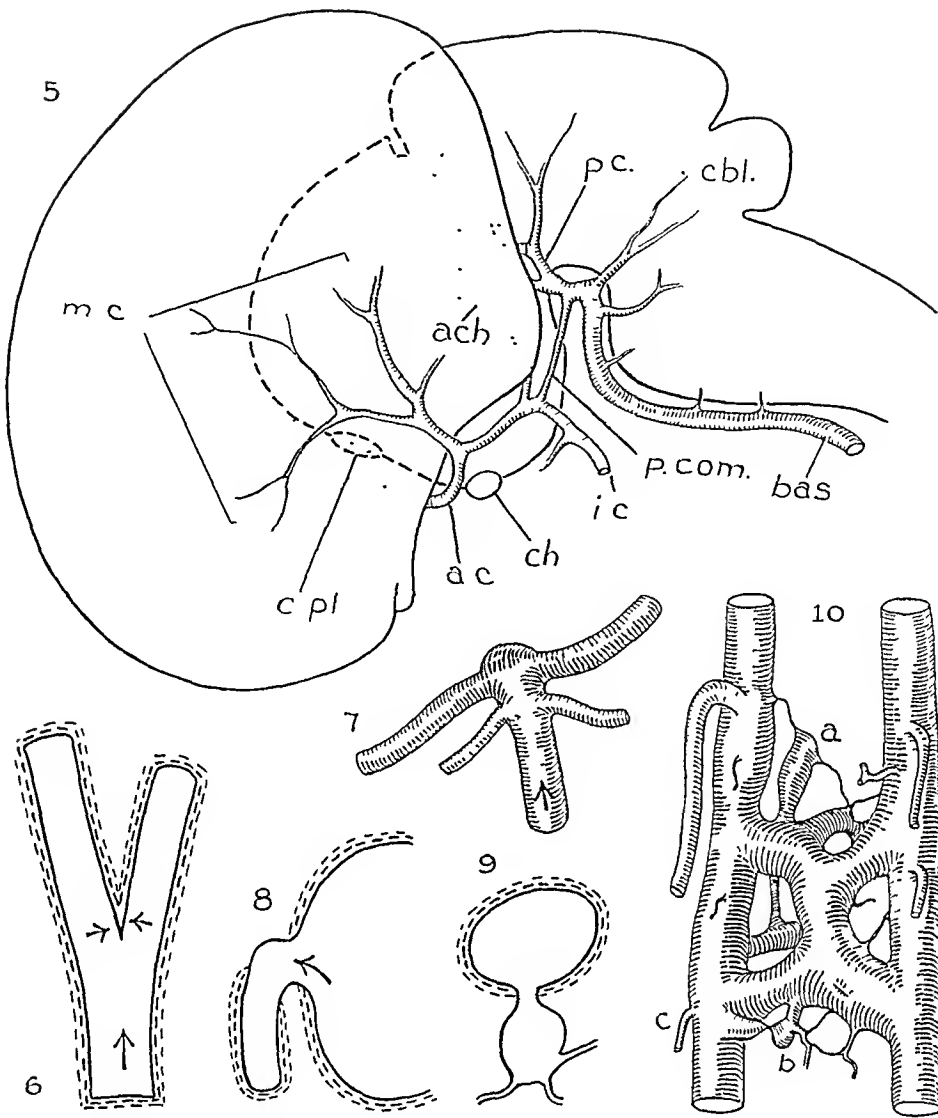


Fig. 5.—Cerebral arteries of a human embryo of 45 mm. at the beginning of the third month.

Fig. 6.—Diagram to show the probable direction of pressure at the forks of an acute bifurcation, with effect on muscle formation.

Fig. 7.—Terminal bifurcation of the basilar artery of a human embryo of 42 mm., with an aneurysmal pouch between the widespread posterior cerebral arteries.

Fig. 8.—The case of an artery of Heubner's system arising from the anterior cerebral artery in a woman of 59 years. Note the wide muscular gap with shallow pouch. See text for further comment.

Fig. 9.—Minute aneurysm from the side of the anterior cerebral artery of a human embryo of 45 mm., resulting from irregular degeneration of certain members of a periarterial capillary plexus.

Fig. 10.—Anterior communicating artery of an infant 1 week old, showing complicated plexus formation, with closure of some portions, and two aneurysmal pouches (*a* and *b*) with little or no outlet. An artery of Heubner's system is shown (*c*).

fusion forces before it the pia and with this the anterior choroid artery, which thus changes its course and its angle of origin, as can be followed in figures 3 and 5.

Beyond the point of actual fusion of the two brain walls the membranes are closely apposed, and this gives an opportunity for branches of the posterior cerebral artery, which, as has already been noted, is primarily directed to the diencephalon and midbrain, to sprout in a new direction and supply also the mesial surface of the caudal half of the hemisphere. With the continued growth of the latter, these new vessels assume the dominant role; those to the thalamus and superior colliculus are relegated to the position of minor branches.

In the midbrain the roof produces the corpora quadrigemina (whose bulk has no special influence on the basal vessels) and, below the level of the aqueduct of Sylvius, becomes thickened by the growth of the red nuclei, the reticular formation and the substantia nigra, and by the extension of many of the fibers of the internal capsule as the cerebral peduncles. Much of the thickening thus caused is somewhat lateral, leaving the depression known as the interpeduncular space. The result is the partial obliteration of the deep notch between the forebrain and the hindbrain, seen in the younger embryos. The trunk artery follows this change, and the stems of the posterior cerebral and superior cerebellar arteries of necessity become correspondingly longer without appreciable change in their angle of diversion from the main trunk. The numerous small vessels from the trunk to the basal part of the brain in the region of the notch may, however, suffer much distortion by this change, and even the main trunk vessel, deprived of its normal linear growth, may tend to become tortuous.

The increase in width of the individual hemispheres and of the brain as a whole changes the course of other cranial arteries and, with this, the angle at which they leave the parent stem. The middle cerebral artery runs to the lateral surface of the hemisphere, the anterior artery to the mesial surface. Since their point of origin from the internal carotid artery lies close to the base of the brain, growth in width of the hemisphere spreads them apart until at birth the angle between them is almost 180 degrees. The gradual spreading of this angle can be followed in figures 1 to 5. In the youngest embryo the angle is acute, about 20 to 25 degrees as measured in the sections; at 45 mm. it is over 90 degrees. The two internal carotid arteries and the two posterior communicating arteries are merely carried bodily laterally, but the angle at the junction of the latter two with the single median basilar artery is much increased. This junction takes place topographically, as has been noted, between the origins of the embryonic posterior cerebral and superior cerebellar arteries; in the adult the small section of the original

trunk vessel from the junction to the original root of the posterior cerebral vessel is considered as a portion of the latter artery, the posterior communicating artery becoming a branch. This is due to the increased blood supply from the vertebral arteries after their fusion with the basilar artery, which causes a reversal of flow in the latter and makes the posterior cerebral arteries appear as its terminal bifurcation. The angle between them at their divergence may be as much as 180 degrees in the adult, though in the original pattern the angle between the two arterial trunks was very narrow. Since many cerebral aneurysms occur at the angles between the anterior and middle cerebral arteries and at the terminal bifurcation of the basilar artery, the changes due to growth at these two points are especially significant. The superior cerebellar arteries, since they sprout from the two sides of the median basilar artery, merely change their position in the wall of the parent vessel to accommodate the increasing width of the hindbrain, without essentially altering their angle of emergence.

Another structure whose growth influences the course of one of the main basal vessels is the corpus callosum. This develops, as was shown, by Marchand,<sup>4</sup> in the commissural plate, a thickening in the lamina terminalis or end plate of the median forebrain, shown in figure 5. Through this thickening fibers grow from one hemisphere to the other in two bundles, forming the anterior commissure below, the corpus callosum above. The increasing number of fibers in the latter causes the plate to bulge forward as the genu of the corpus callosum, first recognizable in the fourth month. The originally terminal branch of the anterior cerebral artery, ending in the choroid plexuses, is thus pulled away from this connection and is diverted around the genu to the dorsum of the corpus callosum, where it is in position to send further sprouts to the medial wall of the hemisphere above. As the frontal lobe increases in size, other branches supply the rest of its medial surface, and as the genu enlarges, it pulls the artery bodily forward with it.

The primary and secondary branches of all of the cerebral arteries are submitted to a peculiar type of disturbance. The enlargement of the hemisphere is not accomplished by addition to the two ends of the structure, such as one is accustomed to find, for instance, in the growth of the long bones, but by interstitial growth due to universal increase in the number of nerve cells and fibers. The growth of the hemisphere is most rapid in the early months of fetal life and is retarded later, when the appearance of the convolutions allows the cortex to continue its expansion without adding proportionately to the dimensions of the hemisphere. Still later the bulk is again increased by the progressive myelination of the nerve fibers. The middle and posterior cerebral

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4. Marchand, F.: *Arch. f. mikr. Anat.* **37**:333, 1891.

arteries both approach the hemisphere from the center of its lesser curvature, directed at right angles to its length, their branches spreading fanwise. As interstitial growth proceeds, the branches are spread farther apart. The tendency is noticeable in figures 2, 3 and 5. At birth these angles often approach 180 degrees.

The many small branches that arise from the arteries of the circle of Willis to feed the basal ganglions and subthalamie regions can usually accommodate themselves by lengthening and coiling to any change in position of the stem vessels. The small branches of the anterior cerebral artery to the head of the caudate nucleus are, however, an exception. This artery has been pulled so far forward by the growth of the corpus callosum and the frontal lobe that the anterior perforated space through which the branches entered the brain is left far behind, and the direction of the branches, which originally ran at right angles to the trunk or even pointed forward, is reversed. This effect is most noticeable in the more anterior branches. As a group these branches were described by Heubner<sup>5</sup> in 1872 and are known as Heubner's system; they have been carefully drawn by Aitken<sup>6</sup> and can be recognized in figure 10. They offer another example of the spreading of the angle at arterial forks.

All the bifurcations described as showing angles expanding as the result of the growth of the brain are frequent sites of aneurysms. Turnbull<sup>7</sup> suggested that there is an inherent weakness at these points, and Forbus<sup>8</sup> found at many bifurcations a "medial defect," a small area in the fork of the bifurcation devoid of any muscular coat, though intima, elastica and adventitia remain intact. Such deficient areas were quite frequent not only in the cerebral arteries, whether aneurysms were present or not, but also in the coronary and mesenteric vessels, always at acute angles. Forbus postulated that the internal apex of a bifurcation is the point of greatest pressure from the force of the blood stream and that this occasionally ultimately results in eversion of the unguarded apical area in the form of an aneurysm. He sought to demonstrate his point by the use of glass models so made that a small tube opened into the apex of a fork of a larger tube, continuing the direction of the main trunk, and found that sudden increases of pressure were recorded as greater within the central tube than at any other point in the apparatus. The usual absence of the elastic lamina in aneurysms he attributed to degeneration due to constant stretching of the unsupported tissue.

Later writers did not approve this explanation. Tuthill<sup>9</sup> considered the "medial defects" as embedding artefacts, the widening of slits

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5. Heubner, O.: *Centralbl. f. d. med. Wissensch.* **10**:817, 1872.

6. Aitken, H. F.: *Boston M. & S. J. (supp.)* **160**:1, 1909.

7. Turnbull, H. M.: *Quart. J. Med.* **8**:201, 1915.

8. Forbus, W. D.: *Bull. Johns Hopkins Hosp.* **47**:239, 1930.

9. Tuthill, C. R.: *Arch. Path.* **16**:630, 1933.

between adjacent muscle bundles, the fibers of which may run in different directions and pull apart with shrinkage; Strauss and co-workers<sup>10</sup> substantiated the observations, but the rather common occurrence of the defects as compared with the infrequency of aneurysms "leads us to question Forbus' belief that they are congenital anomalies." These authors thought most cerebral aneurysms due to arteriosclerosis. Glynn<sup>11</sup> also found the "defects" in a large percentage of cases out of all proportion to the few aneurysms encountered, and sought to show that the muscle layer is unimportant in maintaining the strength of a vessel wall since the elastic layer alone, with most of the media scraped away, will withstand pressures much higher than those of hypertension. Richardson and Hyland<sup>12</sup> also found the "defects" but agreed that while they may predispose to and determine the sites of aneurysms there must be a superadded lesion which acts by weakening the elastica locally.

Forbus suggested as the cause of the medial defects that arterial branches may form their own independent coats and that there may be a failure of the two muscular systems to unite, though he added that this theory does not adequately explain why the defect should always be located at the acute angle. Study of many acute bifurcations in embryonic arteries at the period when their muscular coats are being differentiated suggests a different initial cause for the gaps. It is known that certain vessels, such as the cranial veins, which rest immediately against the unyielding surface of the skull, are devoid of most or all of their muscular coat on the adjacent side. Also it has been shown that on the embryonic left pulmonary aortic arch, the future ductus arteriosus, the musculature fails to develop where the vessel is partially encircled by the recurrent laryngeal nerve (Bremer<sup>13</sup>). Apparently the muscularis develops only when the intima requires additional strengthening to withstand the increasing centrifugal force of pulsation; any support will serve. At the acute fork of a bifurcation, at an angle of 20 degrees or so, if the edge at the internal apex is sufficiently sharp, the force of the stream will be readily deflected at the apex, and along the divergent sides will be resolved into two components, one continuing its original course, the other directed centrally, as is shown by the arrows in figure 6. The two adjacent walls will be pressed against each other and will support each other; no musculature will develop until the branches become so far separated that the elasticity of the intima will no longer allow the two walls to touch. From this point onward the

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10. Strauss, I.; Globus, H. H., and Ginsburg, S. W.: *Arch. Neurol. & Psychiat.* **27**:1080, 1932.

11. Glynn, L. E.: *J. Path. & Bact.* **51**:213, 1940.

12. Richardson, J. C., and Hyland, H. H.: *Medicine* **20**:1, 1941.

13. Bremer, J. L.: *Anat. Rec.* **27**:1, 1924.

strengthening musculature is necessary. Sometimes the muscle develops further toward the apex on one branch than on the other, or on a main trunk than on a branch, the naked intimal wall then being supported by the outer surface of the other muscular sheet. An example of such asymmetry is shown at the acute angle of the branch in figure 8. The principle is illustrated at the sharp bow of a rapidly moving boat; the water meeting the rigid slanting surface forms a wave directed laterally. With a blunt bow, on the other hand, like that of a square-bowed scow, the whole force of the water is deflected in a wave thrown directly forward. In the apparatus used by Forbus the apex is broadened by the very insertion of the median tube, which thus feels the whole impact of the center of the stream. His results, therefore, apply to blunt or "tuning fork" angles, which in the embryo do not show the "medial defects."

In the subsequent spreading of a bifurcation or fork in which a "defect" is already present, the wall may remain naked or be strengthened by new growth of muscle from the sides. The latter is by far the more common result. The type of reaction may possibly be decided by the rapidity of the spreading. Two instances of continued absence of muscular support are shown in figures 7 and 8. The former depicts the condition in a human embryo of 42 mm., the beginning of the third month, at the bifurcation of the basilar artery into the two posterior cerebral arteries. The roots of the superior cerebellar arteries are also shown. Opposite the end of the basilar artery, in the axis of the blood stream, is a small dome-shaped swelling, very thin walled as compared with the main vessels. This is almost certainly an aneurysm. At another range of life, in a woman of 59 years, the condition shown in figure 8 was found. This depicts the junction of an artery of Heubner's system with the anterior cerebral artery, as at *c* in figure 10. The branch has completely reversed its direction. One may interpret the conditions found by supposing that the upper (anterior) gap in the musculature developed when the angle was acute forward, that with reversal this portion stretched without developing musculature and that this occurred early enough to allow the formation of a second "defect" when the posterior angle became acute. The point to be stressed at present is that the original gap has remained throughout life unsupported by muscle, though the elastic lamina is continuous. There is a shallow bulge in the thin wall but no true aneurysm. That one had not developed may be due to the lateral position of the branch, away from the main force of the blood stream.

Other aneurysms from the sides of the cerebral arteries, not at recognizable forks, fall into a different category from those just described. These may be explained as derangements of some of the

innumerable minute arteries and precapillary vessels, only visible microscopically, which remain as persistent members of the original capillary plexus in the embryo from which the cerebral system has developed. These vessels are much more numerous than is usually recognized and are not adequately described in the textbooks; they serve as vasa vasorum for the larger vessels or supply the tissues of the local meninges. They arise, usually at right angles, directly from the main arteries, piercing the musculature as endothelial tubes surrounded by a minimal amount of connective tissue, or through larger gaps between the muscle bundles, in which case they assume their own slight musculature in the passage. They often retain their plexiform character just outside the vessel wall, branching in all directions. During early development many of the members of the net degenerate and are lost; usually one or two capillaries persist and enlarge, continuing the main stem. Occasionally all the capillary branches remain small or even undergo late degeneration. In the latter condition the combined outflow from the stem becomes less than the inflow from the parent artery. Such a case is shown in figure 9, from an embryo of 45 mm. The main stem, springing at right angles from the anterior cerebral artery, could receive more blood through its relatively wide entrance than could be transmitted through its three minute branches and has responded by dilating, since its walls are still of only capillary thickness. If this process had continued to adult life, an aneurysm would have resulted directly comparable to one described by Forbus (his no. 4) in the same position, with a narrow pedicle and two or three minute vessels branching from it at recurrent angles. The adventitia found in the wall of his specimen probably represents the usual reaction of the surrounding connective tissue to any expanding structure, a type of capsule formation; the muscularis and elastica had never developed on the original capillary.

Another similar condition is given in figure 4, the enlargement of a portion of figure 3, showing the reduction of the original plexus at the base of the posterior cerebral artery to a single stem. The stump of one of the original roots remains large though attached to only a single shriveled branch. It might well be considered as a presumptive aneurysm. Similar plexuses of tiny vessels, some members of which are still in the degenerative stage, are found not infrequently also at the forks of the larger arteries in the adult on microscopic examination. It is probable, therefore, that some of the aneurysms at the forks may be caused by the late irregular degeneration of these plexuses instead of by the pouching of Forbus' "medial defects." Their origin might be disclosed by the presence of minute vessels springing from some portion of their walls.

Analogous to this group are the aneurysms of the anterior communicating artery. This misnamed structure is seldom a single vessel.



Much more often it is composed of several separate parts, variously connected. De Vries<sup>14</sup> gave diagrams of many patterns found in man, all indicating its derivation from an original capillary plexus. In the embryo and the fetus the plexus is always present. In an infant of 1 week, though to the naked eye the connection appeared as a simple H, microscopic examination revealed the pattern of a three-dimensional net (fig. 10), in which certain members are favored, others reduced to microscopic dimensions and many others probably already lost. At *a* and at *b* conditions have arisen similar to those shown in figures 9 and 4, in which a segment of the plexus has been left with a wide entrance and a smaller exit and therefore has already become pouched and is liable to future aneurysmal enlargement, whether from increasing blood pressure or from an alteration in the direction of the stream as this flows by the open mouth, due to subsequent changes in the pattern or to the progressive coiling of the basal arteries often met in older persons. The latter might explain the late recognition of such aneurysms clinically.

A characteristic of true cerebral aneurysms is the absence of the internal elastic membrane as well as of the muscularis. The "medial defects" of Forbus and of Richardson and Hyland retain elastica in spite of the absence of media. Forbus explains its later disappearance as due to continued overstretching; the other authors see the necessity of a superadded lesion to weaken the elastica locally. Both agree that the resulting aneurysms are thus not strictly congenital, since the elastic tissue is lost after birth. This objection does not hold for some of the minute aneurysms shown in this paper.

Benninghoff,<sup>15</sup> in his review of vasculogenesis in man, showed that the elastic plate is first recognizable in the aorta of embryos of 3.5 cm. but that it is not recognizable in the radial artery until the length of the embryo is nearly 9 cm. He pointed out that the process proceeds centrifugally from the aorta. In the absence of any statement concerning the cerebral arteries one may safely place them nearer the latter in point of time, well beyond the age of the embryos in which were found the conditions shown in figures 4, 7 and 9. These, then, may be taken as presumptive aneurysms in which no elastica is present and therefore as true congenital aneurysms. As to whether these would later have assumed the elastic coat, one may be guided by the ideas of Roux,<sup>16</sup> who postulated that the type of vessel wall depends not on the

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14. De Vries, B.: *Arch. di biol.* **21**:357, 1905.

15. Benninghoff, A.: *Blutgefäße und Herz*, in von Möllendorff, W.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1930, vol. 6, pt. 1.

16. Roux, W.: *Theorie der Gestaltung der Blutgefäße*, in Oppel, A.: *Ueber die gestaltliche Anpassung der Blutgefäße*, Leipzig, W. Engelmann, 1910.

size of the vessel nor on the internal pressure of the blood but on the relative strength of the pulse. This theory has been supported by Benninghoff and Spanner<sup>17</sup> in their description of an acardia, a twin fetus with complete absence of heart. The single umbilical artery was continuous across the surface of the placenta with one of the umbilical arteries of the normal twin. The aorta of the acardia thus became an end artery of the twin with reversed current and had all the characteristics of such an artery, with little elastic tissue. To follow this idea further, a blind pouch lying at the side of the blood stream might well be enlarged by the blood pressure and yet, since it transmits no pulsation, develop none of the usual components of an arterial wall. This same principle may even be applied to the walls of pouches which used to transmit pulsation and therefore acquired arterial coats (as in figure 10) but which lost their distal branches at an early age; under such circumstances the elastic tissue might disappear and allow further dilatation. This sequence would render unnecessary the introduction of a pathologic process or dependence on the doubtful theory that an elastic sheet can be worn out by continued overstretching. The aneurysms would be classed as spontaneous, though due to conditions similar to those leading to true congenital aneurysms.

#### SUMMARY

The cerebral arteries are evolved from a capillary plexus arising from the earliest branch of the primitive aortic arch, which runs along the under side of the brain. From the rostral end of the plexus branches go in front of the bulging hemisphere and over its lateral surface, becoming the anterior and middle cerebral arteries. Another part of the plexus becomes the posterior cerebral artery, covering the diencephalon and midbrain. The anterior artery gains the mesial surface of the frontal lobe; the posterior supplies the similar surface of the posterior lobes as their expansion covers in the diencephalon. All cerebral arteries approach from the lesser curvature of the expanding hemisphere, and the interstitial growth of the latter during fetal life rapidly spreads the forks of their branches. Other growth changes have the same action. If these forks lack the media, the rapid spread may produce local aneurysms.

From all the cerebral arteries and from the main basal trunk smaller branches dip into the brain substance and also supply the meninges. These also form plexuses. Proximal members of such plexuses may enlarge while their distal continuations degenerate and may thus become aneurysmal pouches from the main vessels. Both types may be true congenital aneurysms.

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17. Benninghoff, A., and Spanner, R.: *Morphol. Jahrb.* **61**:380, 1929.

# EPICELLULAR AND PERICELLULAR DEPOSITIONS OF AMYLOID AS THE STARTING POINT OF AMYLOIDOSIS

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Recently Pearson and associates<sup>1</sup> described pericellular amyloid rings in the adipose tissue of the adrenal glands in primary systemic amyloidosis. These amyloid rings were found only in the adipose tissue and not in the adrenal tissue proper. The authors considered such rings as "highly characteristic" of primary amyloidosis, of which only 28 cases have been described. I have observed such deposits of amyloid in the adrenal cortex in secondary, as well as in primary, amyloidosis. Amyloidosis is not a rare disease in Holland and I obtained material for study from the departments of pathology of Dutch universities and hospitals. To detect amyloid the methyl violet, iodine green and iodine-sulfuric acid reactions were used.

Of 12 instances in which amyloidosis of the adrenal gland was found, at least 9 were cases of advanced tuberculosis. In 3 cases the amyloidosis appeared to be primary. In 10 cases the epicellular and pericellular depositis in the adrenal cortex predominated.

CASE 1.—The adrenal glands of a patient with pulmonary tuberculosis and systemic amyloidosis contained much amyloid chiefly in the fasciculate and reticular zones. The amyloid was deposited partly around cells and partly on capillaries. The figure illustrates the appearances in the reticular zone. The pericellular deposit of amyloid is shown clearly. A number of cells are surrounded by amyloid rings, which may be fused with the rings about adjacent cells.

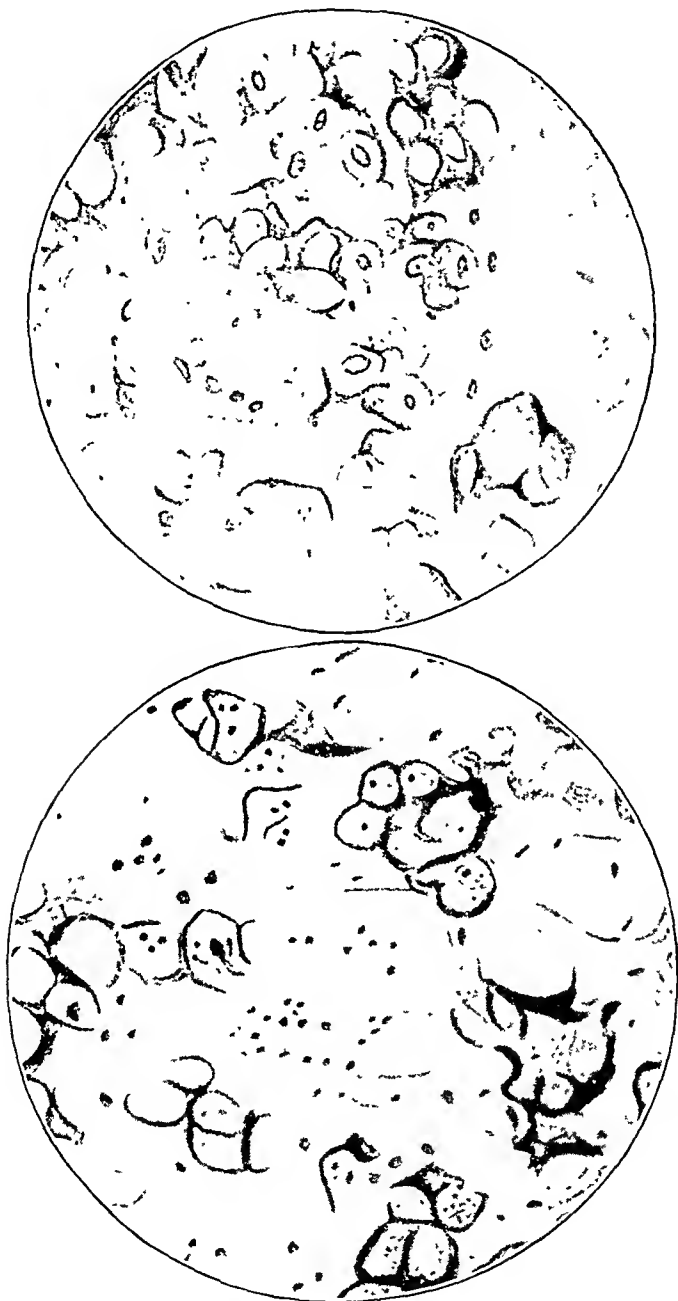
CASE 2.—The adrenal glands of an 18 year old youth with tuberculosis of the lungs, bones and intestines contained large quantities of amyloid in the form of pericapillary and pericellular deposits. The amyloid stained a beautiful blue with iodine-sulfuric acid, especially after fixation in alcohol, suggesting that the amyloid was more "mature" than in other cases.

In only 2 cases was the pericapillary deposit of amyloid preponderant. In the other cases the cellular deposits predominated. Complete rings and shells of amyloid around cells could be observed. The shells did not always have the same thickness at all points. The inner surface of each shell was smooth and less deeply colored with iodine green than other areas. When only part of the cell was covered with amyloid, the deposit was designated as epicellular. The cells entirely enveloped in

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1. Pearson, B.; Rice, M. M., and Dickens, K. La V.: Arch. Path. **32**:1, 1941.

amyloid appeared to undergo gradual atrophy, leaving either empty amyloid shells or solid spheres of amyloid. Some of the empty cavities might have been artefacts due to falling out of cells during the prep-



Upper circle: Pericellular deposits of amyloid. When the objective of the microscope was moved up and down, all rings appeared to be sections of shells. Stained with iodine green.

Lower circle: Amyloid deposits surrounding a number of cells. Note the melting together of the amyloid shells. Stained with iodine green.

aration of the microscopic sections. This possibility seems to be rather slight, however, since I used paraffin sections fixed on cover

glasses. Occasionally cells were found which were smaller than the cavities, and perhaps the atrophy was due more to lack of nourishment than to pressure. That amyloid may be deposited on the inside of the shell is suggested from the fact that the solid spheres of amyloid nearly all had the same diameter, which corresponded to the diameter of the cortical cells. When such spheres had become fused, their contours frequently remained visible.

In other tissues, also, I found amyloid frequently deposited on cells, and it is difficult to understand why the old doctrine of the deposition of amyloid in the interstices of the mesenchymal tissues was not abandoned long ago. In the literature references are made to the deposition of amyloid on cells but without any indication of doubt as to the validity of the old doctrine. Thus, Benecke and Bönning<sup>2</sup> described amyloid deposits in the heart muscles as a walling in of the muscle cells. Hueter<sup>3</sup> wrote that in cartilage the amyloid is deposited first on the walls of the cartilage cells. Spronck<sup>4</sup> in 1919 observed amyloid rings around fat cells. As stated, Pearson, Rice and Dickens<sup>1</sup> described amyloid rings in fat tissue as characteristic of primary systemic amyloidosis. Amyloid deposits on epithelial cells or, more exactly, between epithelial cells and the tunica propria of the renal tubules have been described. The epitelular localization of amyloid in other glands has been observed. In the intima of small veins small deposits of amyloid may occur on epithelial cells, sometimes causing these cells to protrude into the lumen. In connective tissue and other tissues amyloid may be deposited on capillary endothelial cells.

Several points in the localization of amyloid are unexplained by the old doctrine. The pericapillary deposit of amyloid was looked on as the result of transudation of a precursor of amyloid from the blood through the capillary walls. The cardiac valves and the chordae tendineae have no blood vessels, however, and, in spite of that, ramifying amyloid deposits may occur. Here the theory of transudation fails completely. Schmidt<sup>5</sup> noted that the deposits of amyloid in the cardiac valves are covered with endothelial cells. May not this be a deposit of amyloid on the cells of lymph vessels? Unexplained by the theory of transudation is the fact that in arteries, especially the larger, where a stream of transudation can hardly occur, deposits of amyloid are found in the media in the early stages of amyloidosis. Unexplained also are branching amyloid deposits in the arterial media. Schmidt<sup>5</sup> found that in the media of small arteries deposits of amyloid occupy

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2. Benecke, R., and Bönning, L.: *Beitr. z. path. Anat. u. allg. Path.* **44**: 362, 1908.

3. Hueter, C.: *Beitr. z. path. Anat. u. z. allg. Path.* **49**:100, 1910.

4. Spronck: Personal communication to the author.

5. Schmidt, M. B.: *Verhandl. d. deutsch. path. Gesellsch.* **7**:2, 1904.

the position of muscle cells. Here again deposition of amyloid on the muscle cells would explain the situation. A disputed point has been the difference in location of amyloid deposits in connective tissue. In fibrous connective tissue the amyloid is not deposited on the fibers as it is in reticular connective tissue. The explanation seems to be simple: In fibrous connective tissue the fibers are not covered with cells, but in reticular connective tissue the fibers and also the processes are covered with cells, as described by Pekelharing,<sup>6</sup> on which amyloid is deposited.

#### SUMMARY

The deposition of amyloid on cells rather than deposition in the interstices of mesenchymal tissue may be the starting point of amyloidosis.

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6. Pekelharing, C. A.: *Voordrachten over weefselleer*, Haarlem, F. Bohn, 1905, p. 225.

# EPINEPHRINE AND RELATED SUBSTANCES IN HUMAN ARTERIAL WALLS AND KIDNEYS

## THEIR ROLE IN ARTERIOSCLEROSIS

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Arteriosclerosis is often referred to as a vascular condition produced by a "damaging agent" of unknown nature.<sup>1</sup> Little attention has been paid so far to the fact that the body contains a substance which is known to cause severe vascular damage if administered or secreted in abnormally large amounts, namely, epinephrine, the most thoroughly studied among the hormonal substances which are manufactured and secreted by the medulla of the adrenal gland. A very similar, if not identical, substance, sympathin, is liberated by peripheral sympathetic neurons in the vascular walls themselves.<sup>2</sup>

That epinephrine and related substances play an outstanding role in the development of arteriosclerosis and arteriolosclerosis is suggested by the following facts:

- (a) In animals repeated injections of epinephrine hydrochloride produce severe medial changes in arteries, analogous to those found in human arteriosclerosis.<sup>3</sup>
- (b) In rabbits experimental cholesterol lipoidosis of the intima was found to be greatly enhanced by injections of epinephrine<sup>4</sup> and of lipid extracts of the adrenal glands.<sup>5a</sup>
- (c) Epinephrine is absorbed by arterial tissue in vitro.<sup>6</sup>

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1. Duff, G. L.: *Arch. Path.* **20**:81 and 259, 1935. Jobling, J. W., and Meeker, D. R.: *ibid.* **22**:293, 1936.

2. (a) Gaddum, J. H., and Kwiatkowski, H.: *J. Physiol.* **96**:385, 1939.

(b) Cannon, W. B., and Lissák, K.: *Am. J. Physiol.* **125**:765, 1939.

3. Josué, O.: *Compt. rend. Soc. de biol.* **55**:1374, 1903. Hesse, M.: *Virchows Arch. f. path. Anat.* **249**:437, 1924.

4. Danisch, F.: *Beitr. z. path. Anat. u. z. allg. Path.* **79**:333, 1928. Anitschkow, N., in Cowdry, E. V.: *Arteriosclerosis*, New York, The Macmillan Company, 1933.

5. Raab, W.: (a) *Ann. Int. Med.* **14**:1981, 1941; (b) *Arch. Int. Med.* **68**:713, 1941; (c) *J. Clin. Endocrinol.* **1**:977, 1941; (d) *Endocrinology* **28**:325, 1941; (e) **29**:126 and (f) 564, 1941; (g) *Am. Heart J.* **24**:365, 1942; (h) *Exper. Med. & Surg.* **1**: no. 2, 1943; (i) *Endocrinology* **32**:226, 1943.

6. Tatum, A. L.: *J. Pharmacol. & Exper. Therap.* **4**:151, 1913.

- (d) Repeated implantations of adrenal tissue cause vascular changes similar to arteriolosclerosis.<sup>7</sup>
- (e) Arteriosclerosis and arteriolosclerosis, including nephrosclerosis, are common in persons with tumors of the adrenal glands,<sup>8</sup> even in infancy and youth.
- (f) Most patients with pituitary disorders which are accompanied by hyperplasia of the adrenal glands show arteriosclerotic changes.<sup>9</sup>
- (g) Clinical conditions which are intimately connected with arteriosclerotic changes, such as essential hypertension and angina pectoris, have been found to be characterized by abnormally intense temporary discharges of epinephrine and epinephrine-like substances into the blood stream.<sup>5b,c</sup>
- (h) The heart muscles of most persons who died from "hypertensive" and "arteriosclerotic" heart disease were found to contain abnormally high concentrations of epinephrine and of epinephrine-like substances.<sup>5h</sup>

In view of the facts just mentioned it appeared desirable to determine the quantitative concentration of epinephrine and related substances in human arterial walls and kidneys.

#### METHOD AND MATERIAL

Shaw's colorimetric method for the determination of epinephrine<sup>10</sup> as modified by me<sup>5d,e</sup> was used. Other substances, besides epinephrine proper, which participate in the results obtained with this method are related compounds with a catechol nucleus (such as adrenalone, dihydroxyphenylalanine, leukoadrenochrome, sympathin) and ascorbic acid.<sup>5i</sup> The adrenal medulla itself was found to contain relatively large amounts of such substances besides epinephrine proper.<sup>5i</sup>

The term "AC" (from "adrenal catechols") will be used in the tables and chart to designate the total content of chromogenic material described in the foregoing paragraph (i. e., epinephrine, other adrenal catechols and ascorbic acid) on which the colorimetric result in each case was obtained. (In several previous publica-

7. Leriche, R., and Froelich, F.: *Ann. d'anat. path.* **13**:1039, 1936. Hornowski, J.: *Virchows Arch. f. path. Anat.* **215**:280, 1914. Maggi, N., and Mazocchi, E.: *Arch. ital. di chir.* **35**:369, 1933.

8. (a) Paul, F.: *Virchows Arch. f. path. Anat.* **282**:256, 1931. (b) Biebl, M., and Wichels, P.: *ibid.* **257**:182, 1926; *München. med. Wchnschr.* **75**:656, 1928. (c) Kremer, D. N.: *Arch. Int. Med.* **57**:999, 1936. (d) Hegglin, R., and Nabholz, H.: *Ztschr. f. klin. Med.* **134**:161, 1938. (e) Mainzer, F.: *Acta med. Scandinav.* **87**:50, 1935. (f) Moltschanoff, W. J., and Davydowski, J. W.: *Virchows Arch. f. path. Anat.* **274**:606, 1930. (g) Büchner, F.: *Klin. Wchnschr.* **17**:617, 1934. (h) Wells, H. G., in Cowdry, E. V.: *Arteriosclerosis*, New York, The Macmillan Company, 1933. (i) Fuller, R. H.: *Arch. Path.* **32**:556, 1941.

9. McMahon, H. E.; Close, H. G., and Hass, G.: *Am. J. Path.* **10**:177, 1934. Kessel, F. K.: *Ergebn. d. inn. Med. u. Kinderh.* **50**:620, 1936. Raab,<sup>5a</sup>

10. Shaw, F. H.: *Biochem. J.* **32**:19, 1938.



tions<sup>11</sup> that abbreviation was used for "adrenocortical" compounds in the erroneous belief that cortical steroids participate directly in the colorimetric results, owing to compound formation with epinephrine. This conception was later abandoned, however, when it was found that such steroids, although altering the chromogenic and biologic properties of epinephrine,<sup>51,5</sup> do not unite with it in water-soluble compounds.)

The colorimetric readings are expressed in color units per gram of fresh tissue, each of which corresponds to the color intensity of  $10^{-6}$  mg. of pure epinephrine. A rough evaluation of the qualitative composition of the total chromogenic material is possible through determination of the "specific ratio,"<sup>12</sup> the denominator of which (d.s.r.) varies with the relative amount of epinephrine and of the other constituents of the total material. A denominator from 2.00 upward indicates prevalence or exclusive presence of epinephrine (and sympathin); lower figures between 2.0 and 1.0 indicate increasing participation of other, epinephrine-like substances, and an occasional denominator lower than 1.0 is attributable to prevalence of ascorbic acid.

The ascending parts of 42 aortas, 12 renal arteries and 42 kidneys (cortical tissue) were examined within a few hours after autopsy, during which time the tissues were kept in saline solution in the refrigerator. Immediately before chemical examination the arteries were stripped of their adventitia and the kidneys of their capsule. From 400 to 800 mg. of tissue was superficially dried with filter paper, weighed and worked up as described in previous publications.<sup>5d, e</sup> The fact that the material was not available immediately after death possibly accounts for some of the lower figures for total chromogenic material, since a slow loss of color takes place in the dead tissues. The denominator of the specific ratio remains practically unchanged, however.

## RESULTS

*Aorta.*—In table 1 37 aortas of adult persons are grouped according to absence or presence of macroscopically detectable arteriosclerosis. In these two groups, as well as in a few infantile aortas, the majority of the denominators were far above 2.00, indicating the presence of pure epinephrine or of a substance closely related to it, probably sympathin.

The average readings of total chromogenic material (AC in table 2) were about the same in the nonsclerotic and in the sclerotic aortas of adults. However, the presence of chromogenic material other than epinephrine proper and sympathin (indicated by a denominator lower than 2.00) was more frequently encountered in arteriosclerotic aortas, particularly in those with sclerosis of higher degrees. Excessively high concentrations of chromogenic material (above 1,000 color units per gram) were present in 3 aortas of the latter group (cases 26, 31 and 37). The highest value was found in a case in which an adenoma of the adrenal cortex extended into the medulla (case 31).

When all aortas were grouped according to age (table 2), a marked increase of the values for total chromogenic material was noted with age,

11. Raab (footnotes 5 b, c, d, e and f).

12. Shaw.<sup>10</sup> Raab.<sup>51</sup>

TABLE 1.—Cases in Which Aorta Was Studied

Case	Sex	Age	Degree of Arteriosclerosis	Blood Pressure	. AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
Normal Aorta							
1	♂	19	..	126/ 84	83	>4.00	Chronic heart failure
2	♂	20	..	120/ 50	715	>4.00	Chronic heart failure
3	♀	27	..	112/ 74	622	2.20	Carcinoma of uterus
4	♀	38	..	165/ 90	656	>4.00	Uremia
5	♀	39	..	110/ 80	Trace	>4.00	Pneumonia
6	♀	42	..	112/ 84	567	4.00	Peritonitis
7	♀	43	..	210/140	426	>4.00	Chronic heart failure
8	♀	50	..	230/100	610	>4.00	Cerebral hemorrhage
9	♀	51	..	.....	585	1.27	Intestinal hemorrhage
10	♀	51	..	120/?	Trace	>4.00	Appendicitis
11	♀	58	..	130/100	773	1.52	Carcinoma of the breast
12	♀	63	..	90/ 50	625	1.41	Carcinoma of the sigmoid
13	♀	69	..	110/ 65	595	>4.00	Pernicious anemia; pneumonia
14	♀	71	..	112/ 72	518	>4.00	Sarcoma of small intestine
15	♀	78	..	160/ 70	739	>4.00	Chronic heart failure
16	♀	81	..	160/ 72	775	2.45	Chronic heart failure
17	♀	92	..	160/ 72	104	>4.00	Senility
Sclerotic Aorta							
18	♂	37	+	.....	163	>4.00	Cerebral hemorrhage
19	♂	46	+	100/ 80	195	3.80	Cerebral hemorrhage
20	♂	55	+	90/ 52	100	>4.00	Chronic heart failure
21	♂	62	+	.....	686	3.28	Carcinoma of the sigmoid
22	♂	63	+	246/100	151	3.80	Surgical shock
23	♂	69	+	100/ 56	532	>4.00	Pulmonary embolism
24	♂	74	+	142/ 76	548	1.91	Carcinoma of cecum
25	♂	83	+	150/ 62	Trace	>4.00	Pneumonia
26	♂	39	++	238/144	1,278	1.35	Uremia
27	♂	48	++	182/116	58	>4.00	Cerebral hemorrhage
28	♂	58	++	110/ 50	752	1.75	Intestinal hemorrhage
29	♂	58	++	225/130	470	1.56	Uremia; nephrosclerosis
30	♂	63	++	100/ 66	375	2.30	Carcinoma of bladder
31	♂	68	++	138/ 20	1,992	1.43	Adrenal cortical adenoma
32	♂	68	++	100/ 60	15	3.00	Subarachnoidal hemorrhage
33	♂	69	++	140/ 90	Trace	>4.00	Congestive heart failure
34	♂	72	++	218/ 74	751	>4.00	Carcinoma of stomach; adenoma of adrenal medulla
35	♂	76	++	.....	355	1.49	Chronic heart failure
36	♂	81	++	140/ 70	202	3.80	Senility
37	♂	59	+++	"Low"	1,062	1.58	Coronary sclerosis
Infantile Aorta							
38	♂	48 hr.	.....	.....	Trace	>4.00	Hepatic hemorrhage
39	♂	2 mo.	.....	.....	561	.....	Otitis media
40	♂	1 yr.	.....	.....	Trace	>4.00	Marasmus
41	♂	5 yr.	.....	.....	220	3.53	Meningitis
42	♂	7 yr.	.....	.....	82	3.60	Cerebral tumor

\* These figures represent the total colorimetric readings (adrenal catechols, sympathin and ascorbic acid) for which the term AC is used.

† The denominator of the specific ratio (d.s.r.) characterizes the qualitative composition of the total AO material. A d.s.r. from 2.00 upward indicates the prevailing or exclusive presence of epinephrine proper or of sympathin. A lower d.s.r. indicates the presence of other AC compounds.

TABLE 2.—Average Values for Various Groups of Aortas

	Cases	Average Age, Yr.	AC, Color Units per Gm.*	Percentage of Cases in Which D. S. R. † Smaller Than 2.00 Was Found
Degree of arteriosclerosis				
None.....	17	52	493	18
Slight to severe.....	20	62	484	35
Age groups				
0 to 7 years.....	5	2½	173	0
19 to 50 years.....	12	37	507	8
51 to 69 years.....	16	61	575	44
71 to 92 years.....	9	79	444	22
Systolic blood pressure				
90 to 150 mm. Hg.....	19	55	379	27
151 to 246 mm. Hg.....	13	62	616	18

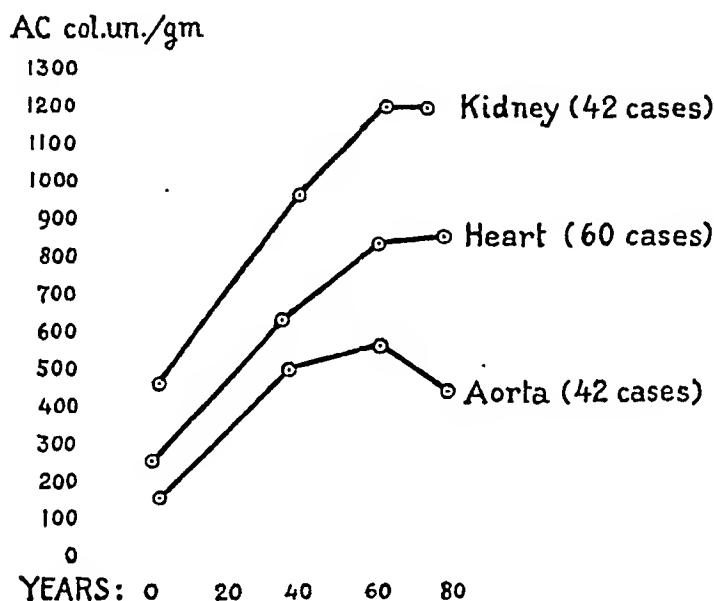
\* See explanation under table 1.

† See explanation under table 1.

the peak occurring between 51 and 69 years, followed by a slight decline (figure). This age curve was roughly paralleled by the frequency of the presence of chromogenic substances other than epinephrine or sympathin.

The aortas of hypertensive persons (table 2) contained on an average larger amounts of chromogenic substances than those of nonhypertensive persons.

*Renal Artery.*—No significant difference of the average concentration of the total chromogenic material was found between 8 nonsclerotic and 4 sclerotic renal arteries (table 3). The values were generally lower than



The increase of the concentration of the total chromogenic material (AC—epinephrine, other adrenal catechols and ascorbic acid) with age in the human aorta, heart and kidney (expressed in color units per gram).

TABLE 3.—Cases in Which Renal Artery Was Studied

Case	Sex	Age	Degree of Arterio-sclerosis	Blood Pressure	AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
Morphologically Normal Renal Arteries							
43	♂	49	..	98/ 70	121	3.80	Carcinoma of cystic duct
44	♂	50	..	130/ 80	39	>4.00	Pulmonary embolism
8	♂	50	..	230/100	61	>4.00	Cerebral hemorrhage
28	♂	58	..	130/100	86	>4.00	Carcinoma of the breast
11	♂	58	..	110/ 50	296	2.00	Intestinal hemorrhage
16	♂	60	..	160/ 72	675	2.46	Chronic heart failure
21	♂	62	..	.....	Trace	>4.00	Carcinoma of sigmoid
31	♂	68	..	138/ 20	784	>4.00	Adrenal cortical adenoma
Average		57	..	142/ 70	258		
Sclerotic Renal Arteries							
24	♂	74	++	142/ 76	387	>4.00	Carcinoma of cecum
35	♂	76	++	.....	595	3.60	Chronic heart failure
20	♂	55	++	90/ 50	Trace	....	Chronic heart failure
25	♂	83	++	150/ 62	47	0.71	Pneumonia
Average		72	....	127/ 63	257		

\* See explanation under table 1.

† See explanation under table 1.

those for the aorta. The highest reading was obtained in a case of adenoma of the adrenal cortex (case 31). With one exception all renal arteries contained pure epinephrine or sympathin.

TABLE 4.—Cases in Which Kidneys Were Studied

Case	Sex	Age	Degree of Nephrosclerosis	Blood Pressure	AC, Color Units per Gm.*	Denominator of Specific Ratio †	Diagnosis
Morphologically Normal Kidneys							
2	♀	20	..	120/ 50	1,368	1.22	Chronic heart failure
6	♀	42	..	112/ 84	835	1.01	Peritonitis
19	♀	46	..	100/ 80	1,684	1.48	Cerebral hemorrhage
43	♂	49	..	98/ 70	767	1.43	Carcinoma of cystic duct
44	♀	50	..	130/ 80	1,065	0.91	Pulmonary embolism
11	♀	58	..	130/100	909	1.19	Carcinoma of breast
21	♂	62	..	.....	641	1.44	Carcinoma of sigmoid
32	♂	68	..	100/ 67	481	0.69	Subarachnoidal hemorrhage
31	♂	68	..	138/ 20	1,795	1.86	Adrenal cortical adenoma
34	♀	72	..	218/ 74	2,947	1.00	Carcinoma of stomach; adenoma of adrenal medulla
1	♂	19	..	126/ 84	1,110	2.27	Chronic heart failure
45	♂	63	..	120/ 80	4,409	1.11	Heart failure; albuminuria ++
33	♂	69	..	140/ 90	579	1.33	Chronic heart failure
24	♂	74	..	142/ 76	1,495	1.60	Carcinoma of cecum; albuminuria +
Kidneys with Arteriosclerosis							
10	♀	51	+	120/ ?	971	1.19	Peritonitis
20	♀	55	+	90/ 62	220	3.70	Chronic heart failure
13	♀	69	+	110/ 65	1,051	1.27	Pneumonia; albuminuria +
16	♀	81	+	163/ 72	2,223	1.06	Chronic heart failure
17	♀	92	+	169/ 72	Trace	....	Senility
18	♂	37	++	.....	461	4.20	Cerebral hemorrhage
7	♂	43	++	210/140	1,621	1.87	Chronic heart failure
27	♀	48	++	182/116	459	1.73	Cerebral hemorrhage
29	♂	58	++	225/130	1,703	1.17	Uremia; albuminuria +
22	♂	63	++	246/100	595	1.88	Surgical shock
23	♂	69	++	100/ 56	1,083	3.11	Pulmonary embolism
35	♀	76	++	.....	439	0.91	Chronic heart failure; albuminuria +
36	♀	81	++	140/ 70	812	1.39	Senility
46	♂	82	++	160/ 92	1,176	1.52	Concussion of brain
25	♂	83	++	150/ 62	549	0.65	Pneumonia
8	♀	50	+++	230/100	427	4.20	Cerebral hemorrhage; albuminuria ++
47	♀	67	+++	160/ 50	293	3.60	Chronic heart failure; albuminuria ++
15	♂	68	+++	160/ 70	739	4.30	Chronic heart failure; albuminuria ++
Infantile Kidneys							
48	♂	9 hr.	.....	.....	76	4.10	Atelectasis
49	♂	13 hr.	.....	.....	315	4.04	Hemorrhage left adrenal gland
50	♂	40 hr.	.....	.....	139	2.02	Atelectasis
38	♂	48 hr.	.....	.....	1,530	0.70	Hepatic hemorrhage
51	♀	7 wk.	.....	.....	408	....	Colitis
39	♂	2 mo.	.....	.....	681	0.95	Otitis media
40	♀	1 yr.	.....	.....	326	1.32	Marasmus
52	♂	1½ yr.	.....	.....	42	3.91	Meningitis
41	♂	5 yr.	.....	.....	386	3.66	Meningitis
42	♂	7 yr.	.....	.....	882	1.47	Tumor of brain

\* See explanation under table 1.

† See explanation under table 1.

*Kidney.*—Forty-two kidneys (including 10 infantile ones) were grouped in the same manner as the aortas (table 4). The qualitative composition of the chromogenic material in the kidney was generally quite different from that in the arterial walls in that the material other than epinephrine proper or sympathin was prevalent in most specimens

deriving from adults. The presence of pure epinephrine or sympathin, although common in infantile kidneys, was confined in the groups of adults almost entirely to those kidneys which showed marked arteriosclerotic changes. The average values for the total chromogenic material increased with age (table 5; figure), and also chromogenic material other than epinephrine was more commonly found in adults than in children, reaching a maximum occurrence in senile persons (100 per cent).

In those cases in which there had been marked albuminuria during life, there was either an abnormally high concentration of total chromogenic material (case 45) or an abnormally high concentration of epinephrine proper in the kidneys (cases 8, 47 and 15). The second highest concentration of the total chromogenic material was found in a case of

TABLE 5.—Average Values for Various Groups of Kidneys

	Cases	Average Age, Yr.	AC, Color Units per Gm.*	Percentage of Cases in Which D. S. R.† Smaller Than 2.00 Was Found
Degree of arteriosclerosis				
None.....	14	54	1,439	93
Slight to severe.....	18	65	810	65
Age groups				
0 to 7 years.....	10	1½	479	44
19 to 50 years.....	10	40	980	70
51 to 69 years.....	14	63	1,203	71
71 to 92 years.....	8	74	1,205	100
Systolic blood pressure				
90 to 149 mm. Hg.....	18	57	1,166	78
150 to 246 mm. Hg.....	11	67	1,131	82

\* See explanation under table 1.

† See explanation under table 1.

tumors of the adrenal medulla (case 34). High amounts of the total chromogenic material were also present in the kidney in a case of uremia (case 29) and in a case of adenoma of the adrenal cortex (case 31).

#### COMMENT

Among the epinephrine-like substances which, together with epinephrine proper, sympathin and ascorbic acid, participate in the color reaction used in the determinations discussed in the foregoing section there are catechol compounds which exert certain pharmacodynamic effects on the cardiovascular system<sup>13</sup> and which are eagerly absorbed by the heart muscle.<sup>5f,1</sup> Their possible effects on arterial and renal morphologic structure have not yet been studied. However, the abnormally high concentrations of epinephrine-like catechols not identical with epinephrine which were almost regularly found in the blood<sup>5b</sup> and the

13. (a) Tainter, M. L.: J. Pharmacol. & Exper. Therap. **40**:43, 1930. (b) Tani, S.: Folia pharmacol. japon. **13**:393, 1932. (c) Oster, K. A.: Nature, London **150**:289, 1942.

heart muscle<sup>51</sup> of uremic patients are paralleled by particularly severe myocardial lesions in uremia.<sup>14</sup> This seems to indicate that other, epinephrine-like substances may be at least as detrimental to cardiovascular structural integrity as epinephrine itself.

The conversion of epinephrine and of its precursors into other pharmacodynamically vasoactive compounds takes place through the action of certain enzymes (amino oxidases, catecholases and so on<sup>15</sup>). Hormonal steroids, particularly those having their origin in the adrenal cortex, modify both the chromogenic and the biologic properties of epinephrine.<sup>16</sup>

With these facts in mind, it will be realized that the amounts and distribution of epinephrine, sympathin and other epinephrine-like substances in arterial walls, kidneys and other tissues will depend not solely on the intensity of adrenal secretion but also on a variety of local neurohormonal and enzymatic processes, presence of certain lipids and other factors. The degree of the destructive effect on vascular tissues will probably likewise be influenced by these factors.

Nevertheless, it seems significant that particularly high concentrations of the chromogenic material were found in the arteries and kidneys of persons with adrenal tumors.

The presence of pure epinephrine or sympathin is much more common in the tissue of the aorta and the renal artery than in the kidney. This may be due to the fact that epinephrine is excreted through the kidneys in a modified form<sup>17</sup> and perhaps also to the presence of lipids in the kidney<sup>81</sup> which are likely to modify the epinephrine molecule.

Age is a determining factor in the vascular and renal distribution of the total chromogenic material: The lowest average values for the total material were found for the aortas and kidneys of infants and young children and the highest ones for those in the sixth and the seventh decade of life. Also the accumulation of the chromogenic substances other than epinephrine proper or sympathin increases distinctly with age both in arterial walls and in kidneys. Analogous conditions, namely, low total chromogenic material and low nonepinephrine material in infancy and increase of both with age, have been observed in the human heart muscle.<sup>51</sup> The changes in the total chromogenic material of the

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aorta with age may possibly be related to the increasing deposition of lipids in the aortic walls with advancing age.<sup>18</sup>

In arteriosclerotic aortas abnormally high values both for the total chromogenic material and for the accumulation of nonepinephrine material were encountered more frequently than in nonsclerotic aortas. Here again the modifying effect of steroids on properties of the epinephrine molecule may be of importance, as well as degenerative changes in the sympathetic ganglions which furnish the nerve supply to the arteriosclerotic vascular areas.<sup>19</sup> Experimental degeneration of sympathetic fibers was found to alter the chromogenic properties of epinephrine or of sympathin in the heart.<sup>20</sup>

Conditions in the arteriosclerotic kidneys were different from those in the aortas so far as the average total chromogenic material was somewhat lower in the sclerotic kidneys than in the morphologically normal ones. Only the infantile and the sclerotic kidneys contained pure epinephrine in a significant number of instances. Analogous conditions were observed by me in the human heart; pure epinephrine was found almost exclusively in infantile hearts and in those of persons who had died from cardiac failure with myocardial hypertrophy and degeneration.<sup>5b</sup> Thus, the behavior of the prevailing muscular renal arterioles in regard to storage and formation of the chromogenic material seems to resemble more that of the heart muscle than that of the elastic fibrous aorta.

Marked albuminuria seems to be connected with high renal concentrations of either the total chromogenic material or epinephrine proper. Experimental albuminuria has been produced by the administration of epinephrine.<sup>21</sup>

In view of the fact that epinephrine elevates the renal threshold for excretion of sugar,<sup>22</sup> the high concentrations of epinephrine observed in infantile and sclerotic kidneys may be regarded as responsible for the high

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dextrose threshold which has been found to be characteristic of infants<sup>23</sup> as well as of diabetic patients with nephrosclerosis.<sup>24</sup>

No clear relations were observed in regard to the concentration of total chromogenic material in the arteries and kidneys of nonhypertensive and hypertensive persons except for a somewhat higher content of the chromogenic material in the aortas in the latter group.

#### SUMMARY AND CONCLUSIONS

Epinephrine and epinephrine-like substances (adrenal catechols) were determined colorimetrically in human aortas, renal arteries and kidneys.

Infantile tissues contained the lowest total amounts of chromogenic material. It consisted almost entirely of epinephrine proper or sympathin.

With advancing age, increasing amounts of other, epinephrine-like substances, similar to those produced by the adrenal medulla, were found to accumulate in vascular walls and kidneys.

Abnormally large concentrations were observed in the vessels and the kidneys of persons with adrenal tumors.

Sclerotic aortas contained high concentrations of chromogenic material other than epinephrine proper more frequently than normal aortas.

In arteriosclerotic kidneys, on the other hand, pure epinephrine or sympathin was more commonly encountered than in morphologically normal kidneys. In this respect arteriosclerotic kidneys resemble the failing hypertrophic and damaged human heart.

In cases of marked albuminuria the renal concentrations of the total chromogenic material or of epinephrine proper were high.

The role of adrenal hormones and sympathin as intrinsic damaging agents in the origin of arteriosclerosis is discussed.

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# EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

## VII. CHRONIC NICOTINE POISONING IN RATS AND IN DOGS

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NEW YORK

In the study of chronic nicotineism, especially as to its arteriosclerotogenic effects, uncertainty still prevails concerning the causative mechanism of the various vascular lesions observed. The controversy revolves about the question whether nicotine exerts a direct vasopressor action or an indirect one through influencing the vasomotor centers of the medulla oblongata or through causing a release of epinephrine from the adrenal glands (Straub and Amann<sup>1</sup>; Cannon, Aub and Binger<sup>2</sup>; Dale and Laidlaw<sup>3</sup>; Eichholtz<sup>4</sup>; Kosdoba<sup>5</sup>; Thienes, Lombard and Lesser<sup>6</sup>; Stätlander<sup>7</sup>), or elicits an allergy with the vascular system as the shock organ. The solution of this problem has been complicated by the fact that experimental studies on animals showed that degenerative arterial lesions could be elicited readily in rabbits by repeated subcutaneous, oral or respiratory introduction of nicotine or nicotine-containing agents (Josué<sup>8</sup>; Gouget<sup>9</sup>; Boveri<sup>10</sup>; Adler and Hensel<sup>11</sup>; Adler<sup>12</sup>; Baylac<sup>13</sup>; Lesieur<sup>14</sup>; Guilan and Gy<sup>15</sup>; von Zebrowski<sup>16</sup>;

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(Footnotes continued on next page)

Gotsev<sup>17</sup>; Lee<sup>18</sup>; Schmiedl<sup>19</sup>; Kosdoba<sup>5</sup>; Krylow<sup>20</sup>; Sstarokadomsky<sup>21</sup>; Miller<sup>22</sup>; Papadia,<sup>22</sup> and others), while the arteries of rats similarly treated remained intact (Staemmler<sup>22a</sup>; Thienes and Butt<sup>23</sup>; Wilson, McNaught and DeEds<sup>24</sup>) in spite of the appearance of adenomatous proliferations in the medulla of the adrenal gland (Staemmler). This observation led Staemmler to the conclusion that rats are refractory to epinephrine and nicotine in vascular respects. The situation was further confused by the claim of McCormick<sup>25</sup> that subcutaneous administration of epinephrine prior to administration of nicotine to rabbits protects against fatal doses of this alkaloid. This contention was disputed in turn by Haag and Fisher<sup>26</sup> and Kin,<sup>27</sup> who reported that epinephrine accentuates the toxicity of nicotine.

The present investigations were undertaken in an attempt to clarify several aspects of the causative and protective mechanisms involved in the production of chronic nicotineism and its vascular effects.

#### EXPERIMENTS

Six mongrel dogs, approximately 3 months old and weighing from 2.3 to 3.6 kilograms, and 150 male and 30 female rats, 3 months old and weighing from 150 to 175 Gm., were used in these experiments.

The dogs as well as 30 male and 30 female rats received subcutaneous injections of solutions of chemically pure nicotine alkaloid (Eimer and Amend) only, at the rate of five injections a week. The dogs were given 0.7 cc. of an aqueous 1:1,000 solution of the nicotine alkaloid for one week, 1 cc. for four weeks, 2 cc. for three weeks, 3 cc. for three weeks, 5 cc. for four weeks, 5 cc. of a 1:660 solution for four weeks and 5 cc. of a 1:225 solution for two weeks. From then on, a 3 per cent solution of nicotine in peanut oil was used, and 1 cc. of this agent was given for three weeks and 2.5 cc. for five weeks. Four of the 6 dogs died during the first

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month of the experiment from intercurrent infections. The 2 surviving dogs were killed for histologic study at the end of an experimental period of ten months. The two series of rats which received nicotine alkaloid only were given 0.2 cc. of an aqueous 1:1,000 solution of this substance for a period of four weeks, 0.5 cc. for four weeks, 1 cc. of a dilution of 1:660 for four weeks, 1 cc. of a dilution of 1:225 for eleven weeks and 1 cc. of a dilution of 1:100 for five weeks. The surviving animals were killed for histologic study at the end of an experimental period of approximately eight months.

The remaining 120 male rats were divided into four series of 30 animals each. In addition to the treatment with nicotine as outlined in the foregoing paragraph, the following medication was given: One series of rats received, with their daily ration of 10 to 15 Gm. of McCollum's stock diet, 0.2 Gm. of ascorbic acid and 0.2 Gm. of cystine. A second series received, immediately after each injection of the nicotine solution, a subcutaneous injection of 0.1 cc. of a suspension of epinephrine in peanut oil (1:10,000). A third series had as supplementary treatment a subcutaneous injection of a solution of desoxycorticosterone acetate in peanut oil (1:2,500). (The desoxycorticosterone acetate was donated by Mr. E. H. Bobst, Roche-Organon, Inc.) The fourth series received a suspension of acetylbetamethylcholine chloride (mecholyl chloride) in peanut oil (1:5,000), 0.25 cc. of this material being injected subcutaneously directly after each introduction of the nicotine solution. After four weeks the concentration of the suspension of mecholyl chloride was increased to 1:400 and was kept at this level to the end of the experiment. All experiments were terminated after eight months, when the surviving rats were killed.

## RESULTS

*Symptoms.*—Spastic convulsions developed in all rats within two minutes after each injection of the solution of nicotine. The convulsive state, which lasted about two to five minutes, was followed by a period of exhaustion and stupor, during which the hindlegs were paralyzed and the respiration was considerably increased. In rats which received an injection of a suspension of epinephrine in oil immediately after the administration of nicotine the onset of the convulsions was hastened and their degree was accentuated. However, preceding this reaction there was a stage of extreme excitation, during which the rats attempted to jump out of the container. The final reactive phase was again one of deep depression. The animals of this series did not gain in weight to any marked degree but remained rather small and skinny. They had rough fur and a haggard look. Their feet were cold and cyanotic to a higher degree than those of any of the other series.

The rats of the nicotine-mecholyl chloride series exhibited a similar primary excitation, during which their eyes became dark red and protruding and shed blood-tinged tears (chromodacryorrhexia). This phase lasted about ten to fifteen minutes and was followed by convulsions and finally by depression. The rats subjected to injections of solutions of nicotine and desoxycorticosterone acetate reacted like animals given nicotine only. In the rats which consumed a diet with additions of cystine and ascorbic acid, the onset of the convulsive stage was delayed, so that it occurred usually not before ten to fifteen minutes after the injection of the solution of nicotine, and the attack was less severe in character and shorter in duration than in animals kept on a stock diet.

The dogs usually vomited after the injection of a solution of nicotine and were either unable to walk or had lost proper control over their legs.

*Mortality Rates.*—These differences in symptomatic reactivity among the various series are reflected in part in their respective mortality rates, which are

presented in figure 1. The curves show clearly that the mortality rate for the animals which received the combined nicotine-epinephrine treatment was the highest, being closely followed by that of the series given the nicotine-desoxycorticosterone acetate and that of the series given the nicotine-mecholyl chloride treatment. The mortality curve of the female rats treated with nicotine only showed, following the eighteenth week of treatment, an abrupt break after having exhibited up to that time relatively low mortality. The increase in mortality among the female rats after the eighteenth week was so great that the mortality curve ultimately matched those of the series given the nicotine-epinephrine and the nicotine-mecholyl chloride treatment. This behavior contrasts sharply with that exhibited by the male rats which received injections of solution of nicotine only. These showed a uniform mortality rate throughout the entire course of the experiment and had a considerably lower total mortality than the female rats identically treated, as 10 per

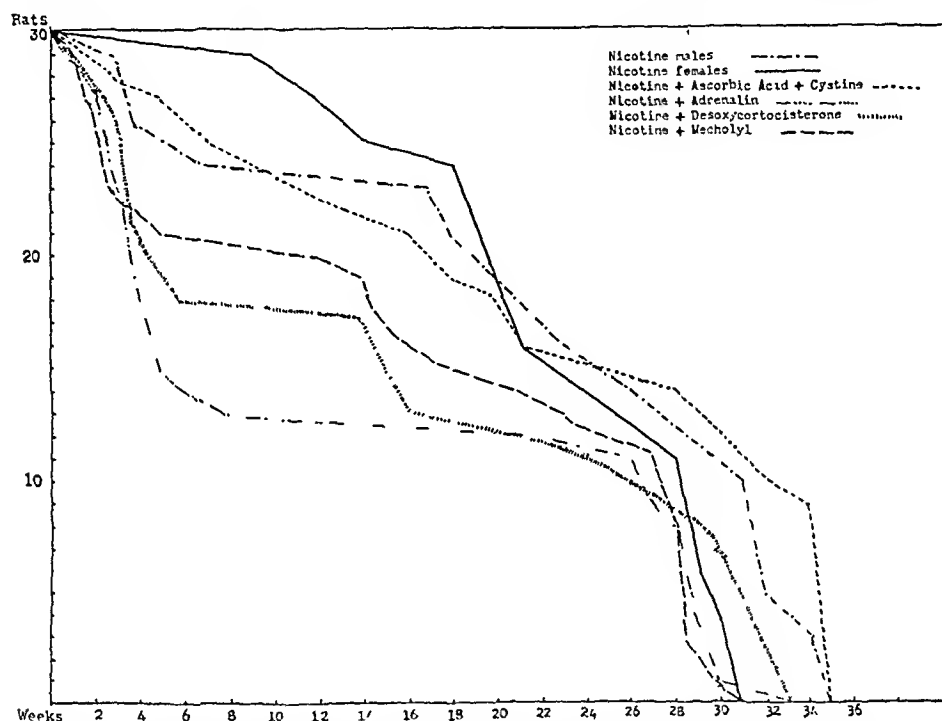


Fig. 1.—Mortality rates of the several experimental series of rats.

cent of the male rats were still alive at the end of the thirty-fifth week. However, by far the lowest mortality rate prevailed among the rats which received cystine and ascorbic acid with their diet. This tendency was particularly manifest during the latter part of the experimental period and was shown by the fact that at the end of the experiment one third of the original number of rats belonging to this series were still alive.

*Pathologic Anatomy of Animals Given Nicotine Only.*—(a) Dogs: Of the 4 dogs which died during the first four weeks of the experiment, 1 had a few hyaline thickenings in the media of the renal arterioles; 3 had marked edema, and 2 a few focal hyalinizations, in the media of the aorta and of the larger elastic arteries. The lungs of these 4 dogs showed extensive purulent and hemorrhagic infiltrations. The adrenal glands showed medullary congestion.

Similar changes in the adrenal glands were found in the 2 dogs which survived for ten months. The thyroid glands of these animals consisted of medium-sized

follicles lined by a single layer of flat epithelial cells and filled with a solid, pink-stained colloid. The anterior lobe of the hypophysis was composed mainly of eosinophilic cells. The myocardial arterioles not infrequently showed thickened and hyaline walls and a proliferated endothelium arranged in palisade formation. Similar lesions were seen in the renal arterioles. The renal artery of 1 dog exhibited a small mushroom-like hyaline thickening of the intima (fig. 2*A*). One dog had similar fibrohyaline intimal deposits in the aorta, while the other dog had a large hyaline scar in the media (fig. 2*B*). In both dogs the inner part of the media of the aorta was edematous. Small scattered perivascular hemorrhages and edema, affecting mainly the vessels at the floor of the fourth ventricle, and some endothelial proliferation of the arterioles were observed in the brain.

(b) Rats: In both the male and the female rats the adrenal medulla was congested and consisted of large juicy cells. In a few instances these extended into the cortex. The adrenal medulla of 1 male rat contained a few giant cells with multiple nuclei. The cortex was relatively narrow. A small extracapsular cortical adenoma was found in 1 male rat. These changes were most pronounced in animals which died during the latter part of the experiment. The testes in 7 of 19 male rats studied histologically showed some kind of degenerative lesions. These affected, however, only exceptionally the entire testicular parenchyma, being restricted in general to small, subcapsularly located areas in which the spermatogenic epithelium was degenerated, where spermatid giant cells were present and where some tubular lumens contained calcified necrotic debris.

Some arterioles of the brain, heart, kidneys and lungs showed an edematous and hyaline thickened media, which occasionally contained focal accumulations of large round or oval nuclei (fig. 2*C*). Perivascular hemorrhages and glia cell infiltrations as well as focal degenerations of the nerve substance were found in a few instances in association with the aforementioned vascular lesions. Endothelial proliferations of the arterioles were exceptional. The aorta was always normal except for some medial edema. Subintimal and medial polypous or trabecular calcifications were noted in the pulmonary artery and its branches in 3 of 29 male and female rats studied. The lungs were often congested and contained more or less extensive hemorrhages. Purulent bronchitis and bronchopneumonia were observed in several animals. Marked congestion of the spleen, the liver and the kidneys was a common feature.

*Pathologic Anatomy of Rats Given Nicotine plus Other Substances.*—(a) Nicotine plus Cystine and Ascorbic Acid: Plump processes of medullary tissue extended not infrequently into the cortex from a congested and relatively large adrenal medulla consisting of juicy cells. In 1 instance a small cortical, subcapsularly situated adenoma and two extracapsular cortical adenomas located nearby in the pericapsular fat tissue were found, while in a second case the adrenal medulla contained two small areas of atypical neurogenic tissue composed of large ganglion cells, glia cells, nerve fibrils and a few small cysts (fig. 3*A*). The testes of 10 of the 20 rats studied showed degenerative changes in the spermatogenic epithelium, affecting usually only smaller areas and involving sometimes only one gonad.

Degenerative vascular lesions affecting the arterioles of the brain, lungs, heart and kidneys were represented by hyalinization and thickening of the media and were most frequent in animals that died spontaneously during the latter part of the experiment. A large hyaline thrombus with calcification and fibroblastic invasion at the base was found in the auricle of 1 rat. Whereas similar but less severe vascular changes were present in many of the 9 rats surviving to the end of the experiment,



Fig. 2.—*A*, mushroom-like fibrous thickening of the intima in a renal artery. *B*, fibrohyaline intimal thickening of the aorta. *C*, hyalinization of the media of a myocardial arteriole containing swollen round nuclei.

3 of these animals were entirely free from any vascular lesion. Two of the surviving rats exhibited small scars in the renal cortex, which were composed of hyaline glomeruli, interstitial lymphocytic accumulations and cystic tubules. Calcifications in the walls of the pulmonary artery and its branches were found in 10 rats. The internal organs (brain, lungs, liver, spleen, kidneys) often showed hyperemia and edema of a moderate to marked degree. Pulmonary hemorrhages were common.

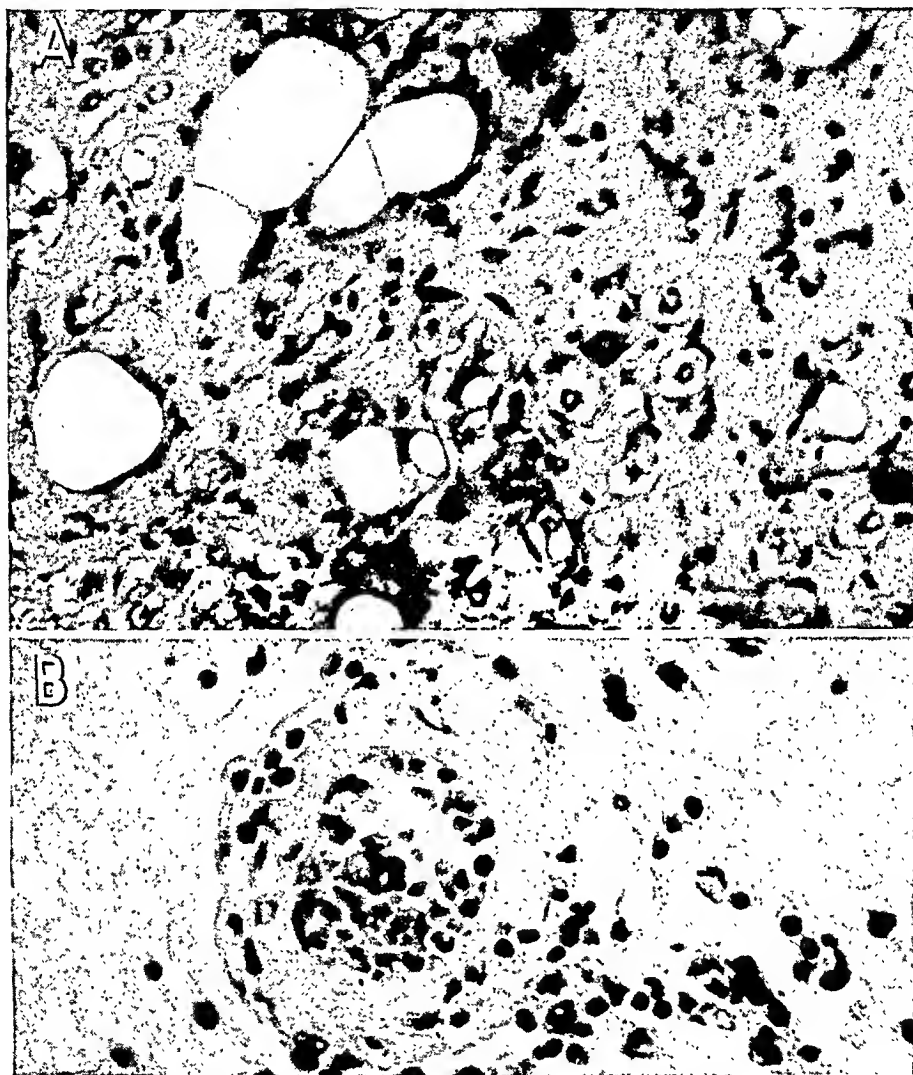


Fig. 3.—*A*, focus of neurogenic tissue in the adrenal medulla, composed of ganglion cells, glia cells and nerve fibrils. *B*, hyalinized and swollen cerebral arteriole with perivascular glia cell infiltration.

(*b*) Nicotine plus Epinephrine: In the rats which died during the first two months of the experiment edema and sometimes hemorrhages in the brain, the lungs, the liver and occasionally the heart were the most striking lesions observed. One rat of this group showed a hemorrhagic erosion of the glandular mucosa of the stomach. Animals which died later usually exhibited adrenal glands with hyperemia and sometimes hyperplasia of the medulla. The medullary cells were large and

juicy. While degenerative and calcifying lesions, occasionally associated with the appearance of spermatid giant cells, were seen in the testes of a few rats, the majority had normal gonads.

Thickened and hyaline arteriolar walls were found in an appreciable number and with increasing frequency in the brain, the lungs, the heart and the kidneys. There was in some vessels focal crowding of hyperchromatic nuclei in the media. Calcifications in the subintima and the media of the pulmonary artery and its branches were seen in only 3 of 22 animals examined histologically. It was noted, on the other hand, that the pulmonary arterioles were not infrequently extraordinarily hypertrophic and showed a swollen and hyaline media. The aorta was normal.

(c) Nicotine plus Desoxycorticosterone Acetate: The adrenal glands of those rats which died during the latter part of the experimental period were large and had a hypertrophic, congested or edematous medulla. Hemorrhage into the cortex was present in 1 rat. The testes of 10 rats of the 25 histologically studied animals exhibited various degrees and types of degenerative and necrotic changes. The severity of these lesions increased with the duration of the experiment.

Similar relations were found in regard to the degenerative lesions involving the arterioles of the brain, the heart, the kidneys and rarely the lungs. These lesions consisted chiefly of thickening and hyalinization of the media. Calcifications of the wall of the pulmonary artery and its branches were present in 6 rats. Edema, hyperemia and hemorrhages were noted in the brain, the lungs, the liver and the spleen. The renal glomeruli of several rats exhibited peculiar hydropic or hyaline swelling or albuminous degeneration of the cellular and interstitial structures, with the swollen and pale blue-stained nuclei of the capsule scattered on the surface and within the homogeneous masses.

(d) Nicotine plus Mecholyl Chloride: The adrenal glands always showed marked medullary congestion. The medulla was composed of large juicy cells, which in several instances were infiltrating the cortical tissue. Nine of a total of 19 histologically studied rats showed some kind of testicular degeneration. Small atrophic and fibrous areas were present in the pancreas in 2 rats, which were the last ones to die in this series. One of these rats had a mild leukocytic infiltration in the androgenic zone of the adrenal glands.

In a minor number of rats the arterioles of the brain, the heart, the lungs and the kidneys showed some hyalinization and thickening of the media. However, the incidence of these lesions was much lower than that of the previously recorded series. One rat had small calcified foci in the wall of the pulmonary artery just above the base of the heart, while another rat had a serous exudate beneath the aortic intima. In 9 instances the pulmonary artery and its branches contained calcifications in the subintima and the media. Perivascular hemorrhages of the brain were found in 12 rats and were associated in 3 with perivascular gliosis and degenerative foci in the nerve substance (fig. 3 *B*). The lungs, the spleen, the liver and the kidneys were usually considerably congested, and pulmonary hemorrhages were common.

#### COMMENT

The observations recorded show that, given excessive doses of nicotine over long periods, dogs and rats will have degenerative lesions of the aorta, the large elastic arteries (pulmonary, renal) and the arterioles of the brain, the heart, the lungs and the kidneys. The pathologic changes in the elastic arteries are characterized by hyaline



thickenings of the intima and by fibrosis, hyalinization and calcification of the media. In the arterioles, on the other hand, they consist of hypertrophy, fibrosis and hyaline thickening of the media and the subintima with occasional proliferation of the endothelial lining. It is noteworthy that in the rats the aorta remained in general free from any pathologic changes, while in the dogs it was regularly involved. The vascular responses obtained in dogs and rats through prolonged administration of nicotine are thus similar to those allegedly following excessive exposure to this agent in man and bear a close resemblance to those previously reported in rabbits. The experimental evidence supporting an arteriosclerogenic action of nicotine is thereby strengthened.

This conclusion receives additional support when proper consideration is given to the morphologic character of the vascular lesions observed. In previous investigations the thesis was advanced that the anatomic type of arteriosclerosis depends on the nature of the causative agent and its particular mechanism of action (Hueper<sup>28</sup>). It was stated that agents which form unstable emulsions with the colloidal solution of the plasma proteins, which are chemically relatively stable and which interfere with the adequate exchange of gases between the interface of blood and vascular wall give rise to the development of atheromatous lesions (cholesterol atheromatosis, polyvinyl alcohol atheromatosis, methyl cellulose atheromatosis, pectin atheromatosis). Disturbances in the quantitative and qualitative relations of the plasma proteins resulting in instability of the colloidal equilibrium of these proteins cause the appearance of fibrohyaline intimal thickenings as well as medial degenerations and calcifications. Increased intravascular pressure as well as vasotonic agents of both the hypertonic and hypotonic types elicit, through the mechanism of ischemic anoxemia or of stagnant anoxemia, fibrohyaline thickenings and intimal proliferations as well as medial degenerations and calcifications. The vascular lesions produced by nicotine in rats and dogs reflect in their morphologic character and their topographic distribution within the vascular tree and vascular wall the vasoconstrictive action of this agent.

It is of interest to note that the toxicity as well as the severity, the number and the distribution of vascular reactions to nicotine could be favorably influenced by exogenous dietary factors (cystine and ascorbic

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28. Hueper, W. C.: *Urol. & Cutan. Rev.* **46**:140, 1942; *Arch. Path.* **3**:1002, 1927; **20**:708, 1935; **31**:11, 1941; *Pharm. Arch.* **11**:49, 1941; *Medicine* **20**:397, 1941; *Arch. Path.* **33**:1, 1942; *Am. J. Path.* **18**:895, 1942; *Arch. Path.* **34**:883, 1942; **33**:267, 1942. Hueper, W. C., and Landsberg, J. W.: *ibid.* **29**:633, 1940. Hueper, W. C., and Ichniowski, C. T.: *J. Lab. & Clin. Med.* **26**:1565, 1941.

acid). However, a relatively high incidence of calcifying vascular lesions of the pulmonary artery and of abnormalities of the adrenal glands was found in this series.

While the addition of the administration of epinephrine to the treatment with nicotine appreciably increased the toxicity of nicotine and thereby accentuated the mortality rate, this management did not result in aggravating significantly the vascular injury as might be expected from the concept of the epinephrinogenic causation of arteriosclerosis in chronic nicotinism. A direct vasculotoxic or indirect cerebral vaso-tonic action of nicotine gains thus in etiologic importance.

A similar accentuation of the toxic effect of nicotine was apparently attained by the addition of desoxycorticosterone acetate.

The combined treatment of rats with nicotine and mecholyl chloride, two vasculotonic antagonists, resulted, on the other hand, in reduction of the degenerative vascular reactions. The relatively high mortality rate observed among the rats of this series was evidently due to the severe circulatory crises following the injection of both nicotine and mecholyl chloride, evidenced not only by the symptomatic reactions recorded but also by the presence of cerebral hemorrhages.

While the increased susceptibility of female rats to nicotine (Staemmler) is confirmed by these experiments, the contention that there is a chemospecific injurious action on the male gonads (Ehrismann<sup>29</sup>; Hoffstaetter<sup>30</sup>) is not supported. The degenerative testicular changes observed in an appreciable number of rats treated with nicotine seem to result secondarily from the circulatory disturbances induced and are probably causally related to more or less prolonged phases of testicular anoxemia, as hematic or vasoconstrictive hypoxemic agents, such as reduced atmospheric oxygen pressure (sojourn at high altitudes), lead, carbon disulfide and carbon monoxide, elicit functional and anatomic testicular degeneration. It may be emphasized in this connection that neither the testicular nor the vascular lesions can be attributed to "physiologic" old age, as their incidence and severity exceed by far those occasionally observed in a series of 100 normal rats of the same age range.

#### SUMMARY AND CONCLUSIONS

Rats and dogs given subcutaneous injections of nicotine over eight to ten months were found to have degenerative lesions in the elastic and muscular arteries and arterioles. The morphologic type of arterial changes produced reflects the nature of the mechanism of action of nicotine (vasoconstrictive ischemic anoxemia).

29. Ehrismann, O.: *Deutsche med. Wchnschr.* **65**:1802, 1939.

30. Hoffstaetter, R.: *Virchows Arch. f. path. Anat.* **244**:182, 1923.

Female rats given injections of nicotine only and male rats given injections of nicotine and epinephrine, nicotine and desoxycorticosterone acetate and nicotine and mecholyl chloride, respectively, showed a much higher mortality rate than male rats receiving nicotine only, particularly male rats given injections of nicotine and kept on a diet containing additions of cystine and ascorbic acid.

Exogenous and, possibly, dietary factors may account in part for the differences in individual susceptibility to nicotine in man and in animals.

The testicular degenerations occurring in an appreciable number of rats treated seemed to be the result of vasoconstrictive episodes of anoxemia.

# STUDIES IN VITRO ON THE PHYSIOLOGY OF NORMAL AND OF CANCEROUS CELLS

## I. THE EFFECT OF HIGH TEMPERATURE AND OF MOCCASIN VENOM ON THE VIABILITY OF RABBIT LYMPHOCYTES AND POLY- MORPHONUCLEAR LEUKOCYTES AS DETERMINED BY THE METHOD OF UNSTAINED CELL COUNTS

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HINES, ILL.

Various types of cells have been extensively investigated from the standpoint of morphology since the days of Virchow. Although the physiology of the cell is as important as its morphology, physiologic studies have been handicapped by the lack of adequate methods. General methods which have been developed for the in vitro study of cell physiology are Warburg's manometric method<sup>1</sup> and tissue culture.<sup>2</sup> Both methods have been found useful but are somewhat difficult to employ in the study of certain physiologic problems.

An attempt was therefore made to find a method which would be as simple as ordinary bacteriologic technic and which could be carried out in any laboratory of pathology. The method that apparently had these qualifications was the counting of the viable cells in a suspension on the basis that imperviousness of a cell to eosin is an indicator of viability. A preliminary report on this procedure was published in 1936.<sup>3</sup>

In the present work the method of unstained cell counts is used to study the effect of elevated temperature and of moccasin venom on the lymphocytes and the polymorphonuclear leukocytes of the rabbit.

### METHODS

To obtain suspensions of lymphocytes, the thymus or the spleen was removed from a normal rabbit which had been killed by an intravenous injection of air. The tissue was thoroughly chopped up with sharp small curved scissors. During this process, a small amount of Tyrode's solution was gradually added. The

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From the Tumor Research Unit, Veterans Administration.

This work is published with the permission of the Medical Director of the Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

1. Dixon, M.: *Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes*, New York, The Macmillan Company, 1934.

2. Parker, R. C.: *Methods of Tissue Culture*, New York, Paul B. Hoeber, Inc., 1938.

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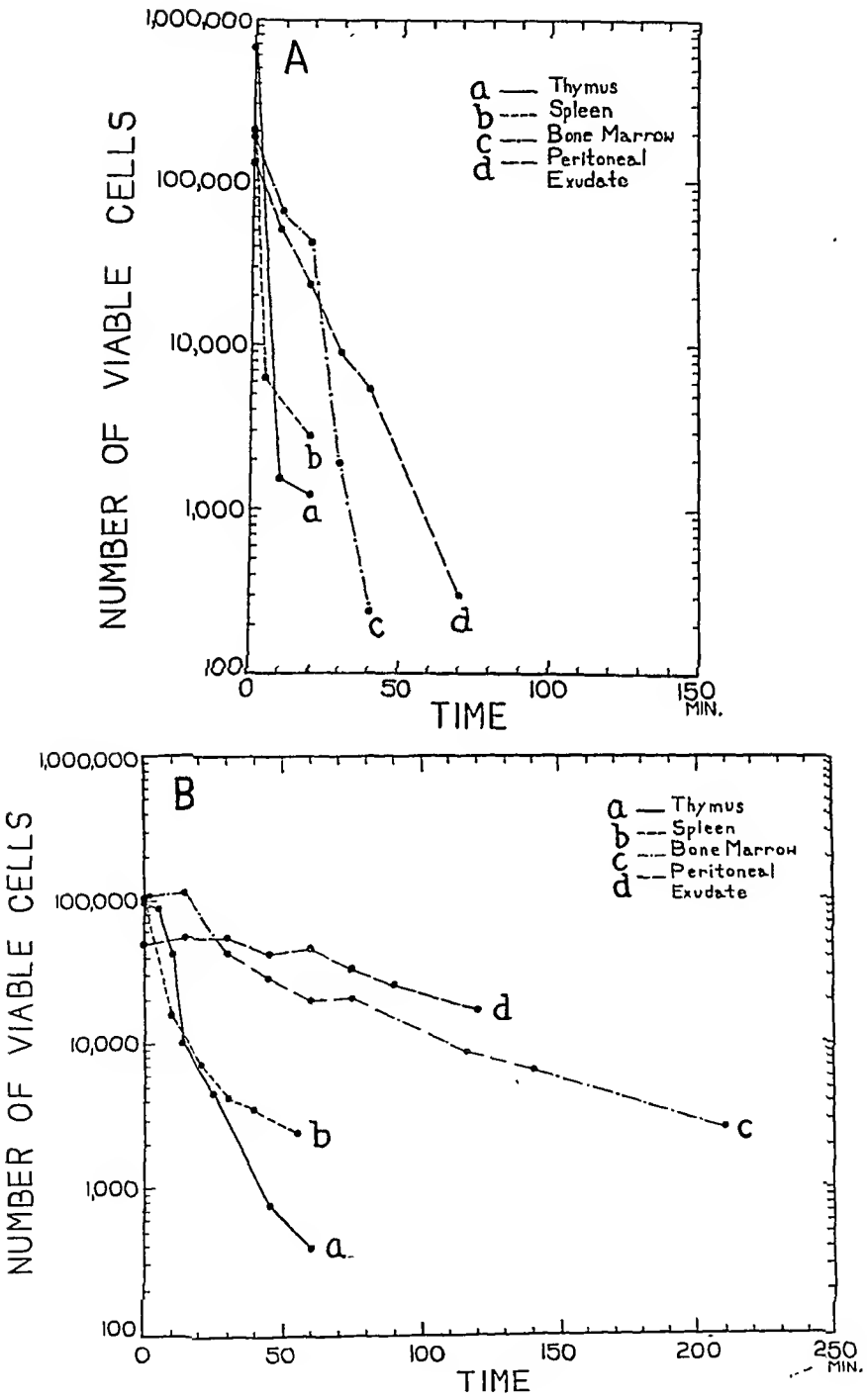


Figure 1

(See legend on opposite page)

minced tissue was filtered in a syringe through 80 gage monel metal wire cloth. On microscopic examination of stained smears, the suspension prepared from the thymus was found to contain isolated cells, most of which were small and large lymphocytes. There were also a few red blood cells. The suspension from the spleen had numerous lymphocytes and very many red blood cells.

The most satisfactory suspension of polymorphonuclear leukocytes was the exudate obtained eighteen hours after an intraperitoneal injection of 200 cc. of a 5 per cent suspension of aleuronat (albuminoid substance and lecithin). The peritoneal exudate thus obtained was centrifuged and the precipitated cells resuspended in a small volume of supernatant fluid, Tyrode's solution or rabbit's serum. Nearly all the cells of the peritoneal exudate were polymorphonuclear leukocytes. A small percentage was large mononuclear cells and red blood cells.

Another suspension of polymorphonuclear leukocytes was obtained from bone marrow. The tissue from the shaft of a rabbit's femur was minced with scissors and filtered through wire cloth. The resulting suspension had different types of cells, but a large percentage was polymorphonuclear leukocytes.

To determine the number of viable cells in these four suspensions, a 1:2,000 solution of eosin in Tyrode's fluid was added (usually 3.8 cc. of dye to 0.2 cc. of suspension). The cells which were stained diffusely red were considered dead, and the cells which were unstained by the eosin were assumed to be viable. The differentiation between the stained and the unstained leukocytes and the red blood cells was nearly always clearcut. The counting of the cells was done in a hemacytometer filled with the suspension-eosin mixture. The results were expressed in cells per cubic millimeter. Most of the counts reported in this paper were performed by technicians.

For the study of the effect of high temperature on cells, the four types of cell suspensions were heated in a water bath maintained at 56, 50 or 45 C. for five to three hundred minutes. The suspensions were contained in small (100 by 13 mm.) stoppered test tubes, each tube having 0.2 cc. of suspension. An eosin solution was added to the heated suspensions, and the stained and unstained cells and red blood cells were counted in a hemacytometer.

#### EXPLANATION OF FIGURE 1

*A*, the effect of exposure to heat at 56 C. on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The ordinates indicate the logarithm of the number of viable cells per cubic millimeter. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus.....	691,000	84,000	25,000
Suspension from spleen.....	204,000	67,000	675,000
Suspension from bone marrow.....	196,000	34,000	149,000
Peritoneal exudate.....	130,000	1,600	46,000

*B*, the effect of exposure to heat at 50 C. on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells.
Suspension from thymus.....	101,000	77,000	6,000
Suspension from spleen.....	105,000	76,000	390,000
suspension from bone marrow.....	103,000	17,000	335,000
Peritoneal exudate.....	51,500	1,500	27,500

Dried moccasin venom was obtained through the aid of Dr. B. W. Carey, of the Lederle Laboratories, Inc. The toxicity of the venom was determined by intradermal injections into rabbits. The minimal intradermal dose was 0.1 cc. of a 1:20,000 dilution, which produced a small reddish black area in twenty minutes.

A mixture of a solution of venom and a cell suspension was incubated at 37 C. in a water bath for various intervals of time; 0.2 cc. of the venom solution was added to 0.2 cc. of cell suspension in a small (100 by 13 mm.) test tube.

After the incubation with venom, the number of viable cells in the cell suspension was again determined. A marked decrease in the number of viable cells as compared with the original count was attributed to the action of venom. Controls were set up to exclude any deleterious effect due to the time of incubation or the Tyrode's solution used.

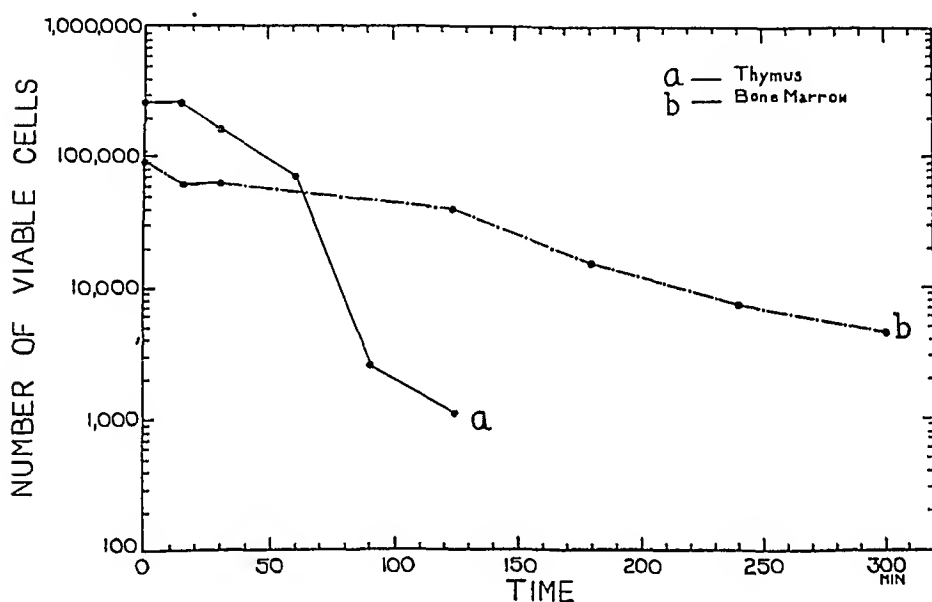


Fig. 2.—The effect of exposure to heat at 45 C. on the number of viable cells in suspensions derived from rabbit thymus and bone marrow. The complete counts on the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus.....	263,000	350,000	4,000
Suspension from bone marrow.....	93,000	63,000	147,000

## RESULTS

*Effect of High Temperature on the Number of Viable Cells.*—Suspensions from thymus, spleen and bone marrow and a peritoneal exudate when exposed to temperatures of 56, 50 and 45 C. suffered a decrease in number of viable cells. The results of typical experiments are shown in figures 1 and 2 and are summarized in table 1. The graphs show the rate of decrease in the number of viable cells for each type of suspension and for each temperature studied, the rate of

decrease being represented by the slope of the curve. The table gives the estimated time required to kill 90 per cent of the viable cells of the original suspension.

*A* and *B* in figure 1 show, in the first place, that the suspensions from the thymus and the spleen were inactivated at approximately the same rate of speed. Ninety per cent of the cells of the two suspensions were killed in less than five or ten minutes at 56 C. and in sixteen minutes at 50 C. (table 1). It seems, then, that the cells in the two suspensions were equally sensitive to the action of the elevated temperature. This finding is consistent with the fact that the nucleated cells of both suspensions are, for the most part, lymphocytes.

It is also seen in *A* and *B* of figure 1 that the rates of inactivation of cells in the peritoneal exudate and the bone marrow suspension were approximately equal. It is estimated that at 56 C., it took twenty-five or twenty-six minutes to kill 90 per cent of the leukocytes in the two

TABLE 1.—*A Comparison of the Effects of High Temperatures on the Cells in a Peritoneal Exudate and in Suspensions of Rabbit Thymus, Spleen and Bone Marrow*

Temperature	Minutes Required to Kill 90 per Cent of the Cells			
	Lymphocytes		Polymorphonuclear Leukocytes	
	Thymic Suspension	Splenic Suspension	Bone Marrow Suspension	Peritoneal Exudate
56 C.	Less than 10	Less than 5	26	25
50 C.	16	16	111	More than 120
45 C.	79	..	226	..

suspensions. It is of interest to note that the mature polymorphonuclear leukocytes of the peritoneal exudate and the immature cells of the marrow had apparently the same rate of death at high temperatures.

The third and most striking point was the difference in the reaction of the suspensions of lymphocytes and polymorphonuclear leukocytes at elevated temperatures. It is seen from figures 1 and 2 that the lymphocytes in the suspensions from the thymus and the spleen were killed at a more rapid rate than the polymorphonuclear leukocytes in the other two suspensions. Table 1 shows, furthermore, that at 56 C. it took less than five or ten minutes to kill 90 per cent of the cells in the thymic and splenic suspensions, compared with twenty-five or twenty-six minutes for the bone marrow suspension and the peritoneal exudate; at 50 C. the corresponding intervals of time were sixteen minutes and two hours; at 45 C. they were seventy-nine and two hundred and twenty-six minutes. It seems, then, that at the three elevated temperatures studied the polymorphonuclear leukocytes of the marrow suspension and the peritoneal exudate were more resistant to the action of heat than the lymphocytes of the thymic and splenic suspensions.



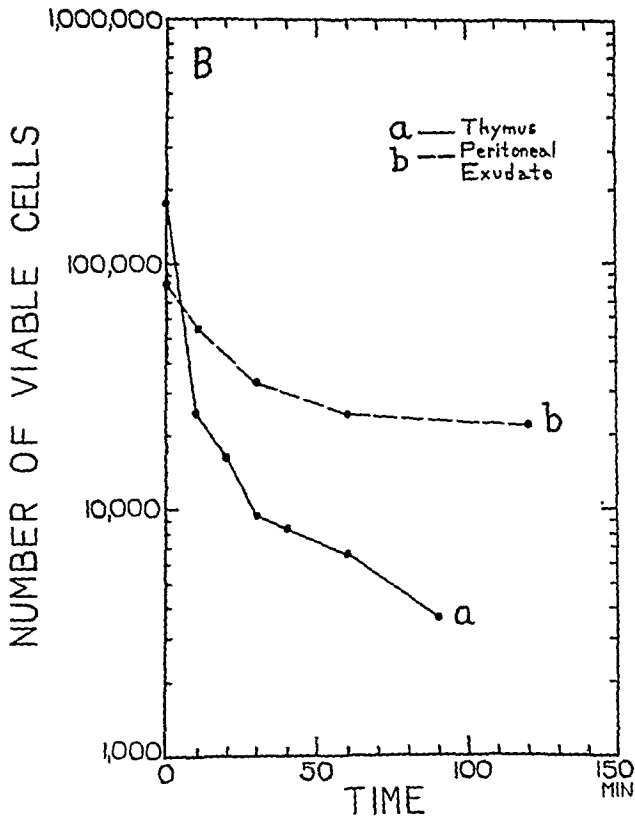
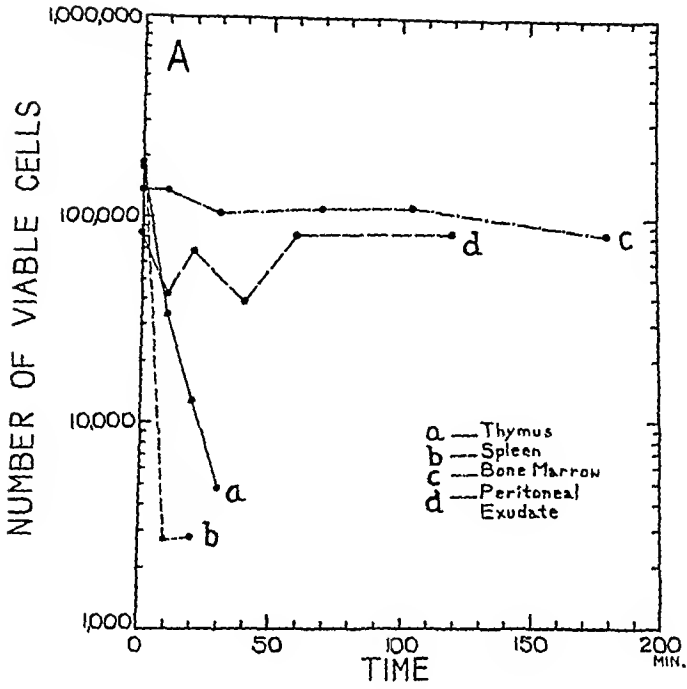


Figure 3

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*Effect of Moccasin Venom on the Number of Viable Cells.*—The effect of adding moccasin venom in 1:2,000 dilution to an equal volume of a cell suspension is shown graphically in figure 3.

It is seen that the number of viable cells (i. e., those which fail to stain with eosin) in the suspensions from the spleen and the thymus decreased rapidly as a result of the action of moccasin venom. In fact, approximately 90 per cent of the cells were dead (i. e., were capable of staining with eosin) in about ten minutes. Since the cells in both suspensions were largely lymphocytes, it seems that this type of cell is killed rapidly by dilute venom.

Figure 3 *A* also shows the effect of this venom in the same dilution on a peritoneal exudate and on a suspension from bone marrow. In contrast to the previous findings, the venom did not produce any appreciable decrease in the number of viable cells even after one hundred and twenty minutes' incubation. As polymorphonuclear leukocytes were predominant in the bone marrow suspension and the peritoneal exudate, it appears that this type of cell is highly resistant to moccasin venom in 1:2,000 dilution.

Further experiments were made to determine the minimal concentration of moccasin venom to which lymphocytes react and the maximal concentration to which polymorphonuclear leukocytes are resistant.

Moccasin venom diluted to 1:10,000, 1:40,000 and even 1:100,000 caused a definite gradual decrease in the number of viable cells in suspensions from the thymus and the spleen. Figure 3 *B* shows the effect of the venom in 1:10,000 dilution on a suspension from the thymus. Only 3 per cent of the viable cells persisted after sixty minutes of incubation with venom in this dilution. In contrast, a concentrated solution of

#### EXPLANATION OF FIGURE 3

*A*, the effect of 1:2,000 dilution of moccasin venom on the number of viable cells in suspensions derived from rabbit thymus, spleen and bone marrow and in a peritoneal exudate. The complete counts of the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus.....	178,000	97,000	9,000
Suspension from spleen.....	188,000	78,000	258,000
Suspension from bone marrow.....	136,000	31,000	640,000
Peritoneal exudate.....	85,000	25,000	31,000

*B*, the effect of 1:100 dilution of moccasin venom on the number of viable cells in a peritoneal exudate as contrasted with the effect of 1:10,000 dilution of venom on the number of viable cells in a suspension derived from rabbit thymus. The complete counts of the original suspensions were as follows:

	Unstained Cells	Stained Cells	Red Blood Cells
Suspension from thymus.....	181,000	148,000	5,000
Peritoneal exudate.....	85,000	25,000	31,000

venom (1:100) caused only a moderate decrease in the number of viable cells of the peritoneal exudate (fig. 3B). After sixty minutes' incubation, 30 per cent of the cells were still viable.

It seems, then, that the lymphocytes of the rabbit are extremely susceptible to the deleterious effects of moccasin venom, but that the polymorphonuclear exudate is almost completely resistant.

*Effect of Moccasin Venom on Agglutination and Lysis.*—To check the findings by another method, a study was undertaken on the effect of moccasin venom on agglutination and lysis of the lymphocytes and the polymorphonuclear leukocytes of the rabbit.

Equal volumes of a cell suspension and moccasin venom were maintained at 37 C. for one hour. The mixtures were then examined. The formation of firm macroscopic clumps which could not be readily

TABLE 2.—*The Effect of Dilution of Moccasin Venom on the Agglutination and Lysis of the Cells in a Peritoneal Exudate and in a Suspension Derived from Rabbit Thymus*

	Tyrode's Solution	Dilution of Moccasin Venom						
		1:100	1:200	1:400	1:1,000	1:2,000	1:4,000	1:10,000
Suspension from thymus								
Agglutination.....	0	0	0	0	A	A	A	0
Lysis.....	0	L	L	L				
Peritoneal exudate								
Agglutination.....	0	0	0	0	0			
Lysis.....	0	0	0	0	0			

A = positive macroscopic agglutination.

L = lysis observed on microscopic examination.

broken up was considered as positive agglutination. Lysis was evidenced by absence of intact cells on smears or on examination in a hemacytometer.

The result of a typical experiment is shown in table 2. It is seen that agglutination occurred one hour after the addition of moccasin venom in dilutions of 1:1,000, 1:2,000 and 1:4,000 to a thymic suspension. With venom in higher concentrations there was no agglutination, the suspensions remaining turbid. Microscopic examination showed, however, the absence of intact cells. Evidently the higher concentrations of venom had caused disintegration or lysis of all the cells. It appears, then, that venom produces both agglutination and lysis of the lymphocytes in a suspension of thymus.

A similar experiment was performed with peritoneal exudate (table 2). No definite evidence of either agglutination or lysis of the polymorphonuclear leukocytes was observed, even in high concentrations of moccasin venom (1:100). It seems that the polymorpho-

nuclear leukocytes of peritoneal exudate are markedly resistant to agglutination and lysis by moccasin venom.

*Effect of Moccasin Venom on Motility.*—The finding of the resistance of the polymorphonuclear leukocyte to moccasin venom is based on indirect methods of determining viability. As the motility of the leukocyte permits direct observation of the viability of the cell, the effect of moccasin venom on the motility of the polymorphonuclear leukocytes was investigated.

Nearly all the cells of a freshly obtained peritoneal exudate were observed to be actively motile and remained so for at least two hours.

Microscopic examination of a mixture of equal volumes of peritoneal exudate and moccasin venom in 1:1,000 dilution showed active motility of the cells during the observation period of two hours. Venom in this dilution had no apparent effect on the motility of the cells.

The addition of a concentrated solution of moccasin venom (1:100) to the peritoneal exudate caused immediate formation of small microscopic clumps of cells. On incubation the cells wandered away from the clumps and became isolated. These processes were photographed and are shown in figure 4. Figure 4 *A*, taken three minutes after the addition of a 1:100 venom solution to a peritoneal exudate, shows a small compact mass. The cells in the periphery are round and do not present any pseudopods. Nine minutes later (fig. 4 *B*) the peripheral cells are elongated and irregular in shape and have moved a short distance from the clump. In twenty-seven minutes after the beginning of the experiment (fig. 4 *C*) all the cells of the clump are isolated from each other, are irregular in shape and have one or more pseudopods.

Further study showed that the cells treated with the 1:100 venom solution were not as active as the cells in control preparations. The treated cells had sluggish motility for thirty minutes. A few of the cells retained that motility for as long as two hours. The high concentration of venom had some injurious effect on the cells, but many of the polymorphonuclear leukocytes remained viable for at least thirty minutes. This finding is in agreement with that obtained by the method of unstained cell counts (fig. 3 *B*).

The work just described was controlled by study of a suspension from the thymus under identical conditions. Venom in dilutions of 1:1,000 and 1:100 caused the lymphocytes in this suspension to disintegrate within sixty minutes, leaving a small amount of amorphous granular debris.

These experiments on motility showed conclusively that moccasin venom in 1:1,000 dilution killed and lysed lymphocytes rapidly but had no effect on the motility and viability of polymorphonuclear leukocytes.

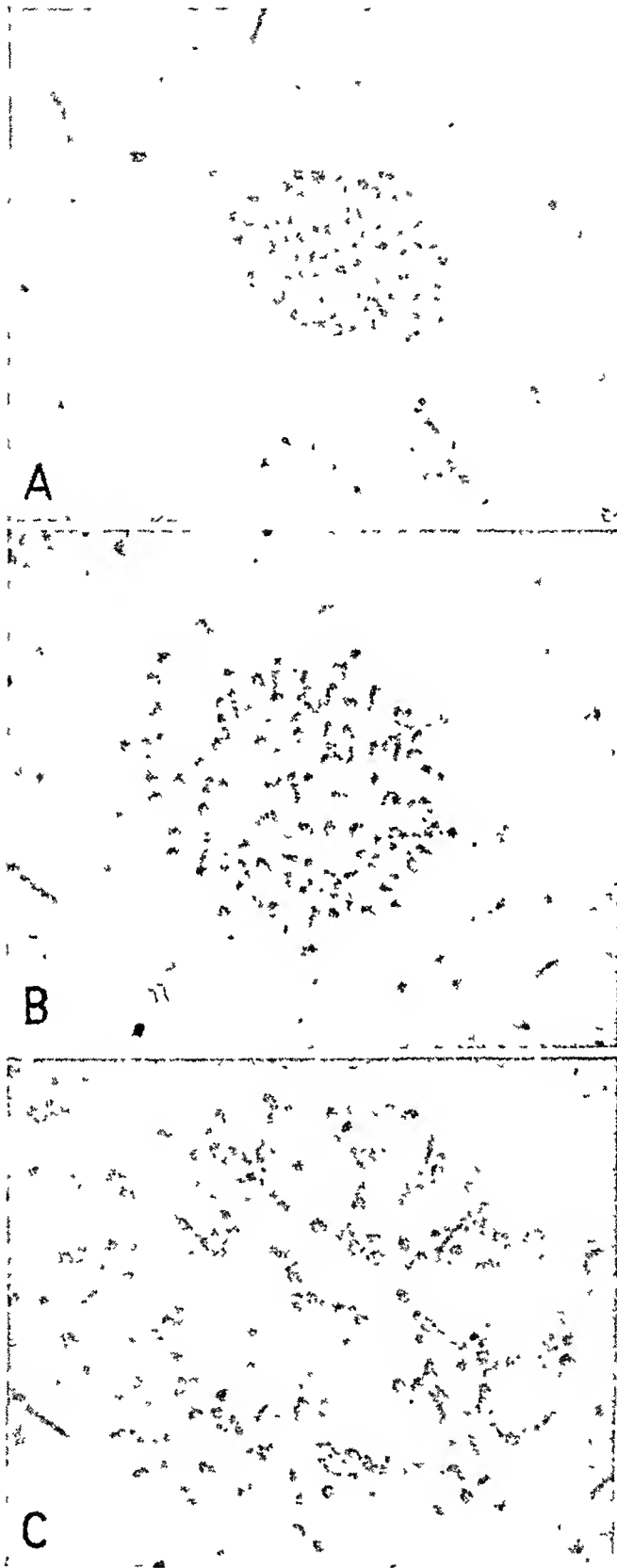


Fig. 4.—The effect of moccasin venom (1:100) on the motility of polymorphonuclear leukocytes in a peritoneal exudate (*A*) three minutes, (*B*) twelve minutes and (*C*) twenty-seven minutes after the addition of the venom.

## COMMENT

*Imperviousness to Eosin as a Measure of Cell Viability.*—What is meant by the death of a cell? How can one differentiate between a dead and a viable cell?

A viable cell may be defined as one having certain essential physiologic functions. Two essential properties, which are probably common to all types of living cells, are known, namely, (1) respiration and (2) selective permeability or impermeability of the cell wall to certain substances. These essential properties should be differentiated from the specialized functions which are found only in certain types of cells. Such specialized functions include (1) mitotic and amitotic division, (2) motility, (3) phagocytosis and (4) contractibility. A cell that has lost one or more of the specialized functions is not necessarily dead. However, a cell that has lost either of the two essential properties, respiration or selective permeability, may be considered dead.

Selective permeability can be determined by means of a suitable dye. Supravital dyes have been used to test for difference in permeability or stainability of dead and living cells. Trypan blue was used by Pappenheimer<sup>4</sup> and neutral red by Achard<sup>5</sup> and Belkin and Shear.<sup>6</sup> It is necessary to use a dye which is water soluble and nontoxic, which gives good differentiation between stained and unstained cells and which rapidly stains cells known to be dead. Eosin seems to satisfy these criteria and has been used in this work.

Viable cells are probably impervious to eosin. A cell which is stained by eosin has lost this impermeability and is in all probability dead. The converse proposition is not, however, necessarily true. It cannot be stated so definitely that a cell which resists staining with eosin is viable. There may be conditions in which the cell loses its property of respiration but retains its resistance or impermeability to eosin. It is also possible that certain chemicals may inhibit the staining of dead cells by eosin. It may, therefore, be useful to apply other tests of viability under certain conditions.

In this work it has been assumed that a cell which takes the eosin stain is dead and one which resists the stain is viable.

*Effect of High Temperature on the Viability of Cells.*—Several investigators have studied the effect of heat on the viability of cells. Clowes<sup>7</sup> showed that a tumor of mice lost its property of transplantability when the cells were exposed to a temperature of 45 C. Pincus and Fischer<sup>8</sup> found that chicken osteoblasts in tissue culture were killed after a six minute exposure to a temperature of 50 C.

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4. Pappenheimer, A. M.: J. Exper. Med. **25**:633, 1917.

5. Achard, C.: Brit. M. J. **2**:1416, 1910.

6. Belkin, M., and Shear, M. J.: Am. J. Cancer **29**:483, 1937.

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In the present study, it took sixteen minutes' exposure to a temperature of 50 C. to kill 90 per cent of the lymphocytes in the thymic suspension and it took more than one hundred and twenty minutes to kill the polymorphonuclear leukocytes in the peritoneal exudate. These results are not directly comparable with those obtained by Clowes and Pincus and Fischer because of the differences in the cells studied and in the methods used for determining viability.

According to the method of unstained cell counts, it appeared that the polymorphonuclear leukocyte is more resistant to heat than the lymphocyte. Experimental factors that may be responsible for this observation are differences in the eosin stainability of heated lymphocytes and polymorphonuclear leukocytes or variations in the milieu of the cells in suspension. It is, however, probable that the observed greater resistance of the polymorphonuclear leukocyte as compared with the lymphocyte is due to morphologic and physiologic differences within the cells.

*Susceptibility of Rabbit Lymphocytes and Resistance of Polymorphonuclear Leukocytes to Moccasin Venom.*—Some toxins are known to act on specific types of cells. Tetanus and botulinus toxins, for example, act on the central nervous system, and tetanolysin and streptolysin on the red blood cells.

In contrast, moccasin venom is reputed to act on many, if not all, types of cells. Flexner and Noguchi<sup>9</sup> showed that moccasin venom causes lysis of the cells of kidney, liver, testis and tracheal epithelium in suspension.

The method of unstained cell counts permitted reexamination of the effects of this venom on lymphocytes and polymorphonuclear leukocytes of the rabbit. The polymorphonuclear leukocytes were found to be highly resistant to the lethal, agglutinative and lytic action of moccasin venom. The resistance of these cells was in marked contrast to the susceptibility of the lymphocytes. The difference in the reaction of the two types of cells is under further investigation.

#### SUMMARY

According to the method of unstained cell counts, the polymorphonuclear leukocytes of the rabbit survive a longer period than the lymphocytes at the elevated temperatures of 56, 50 and 45 C.

Moccasin venom has the capacity of killing, agglutinating and lysing the lymphocytes but has little or no effect on the viability and motility of the polymorphonuclear leukocytes.

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# PINEALOMA

## A CLINICOPATHOLOGIC STUDY OF SEVEN CASES WITH A REVIEW OF THE LITERATURE

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The term "pinealoma" was first suggested by Krabbe,<sup>1</sup> in 1923, for the primary tumors of the pineal body representing neoplasia of pineal tissue. Pinealoma characteristically consists of two types of cells that in many instances show a type of arrangement suggestive of the mosaic pattern observed in pineal tissue at the time of birth. Not all the primary tumors of the pineal body may be classified as pinealoma, however, for Bing, Globus and Simon,<sup>2</sup> in 1938, collected 177 cases of pineal tumor from the literature including, besides those of pinealoma, instances of several types of glioma, instances of a teratomatous type and many cases of tumor not histologically verified. Most of the previous studies of this subject (Horrax and Bailey<sup>3</sup>; Globus and Silbert<sup>4</sup>; Dandy<sup>5</sup>; Baggenstoss and Love<sup>6</sup>) have been concerned mainly with pineal tumors in general and the clinical symptoms produced, because a significant number of pineal tumors in preadolescent boys have been associated with precocious puberty. This has raised the question whether the pineal body is not an endocrine gland. There has been no attempt, however, to collect or to study the tumors with the designation of pinealoma as a group. If the pineal body has a function and its tumor is concerned with the production of precocious puberty, such a study should be of prime importance. Noteworthy histologic contributions to the knowledge of pinealoma have been made by Horrax and Bailey<sup>3</sup> and by Globus and Silbert,<sup>4</sup> although these two groups of investigators do not agree on several fundamental points.

The present report is a clinicopathologic study of 7 cases of pinealoma selected from 14 cases of primary tumor of the pineal body that have been observed in the neurosurgical services of the Barnes Hospital and the St. Louis Children's Hospital during the past twenty years. A pathologic study of tumors diagnosed as pinealoma appeared worth while in the light of the differing opinions because the collection of 7 cases of this rare type of tumor provides a sufficiently large number to allow an adequately detailed clinical and pathologic study. In order to broaden the study and further evaluate the problem of precocious puberty associated with pineal tumors, the previously reported cases of pinealoma have been collected and reviewed.

### REPORT OF CASES

CASE 1.—J. B., a white man aged 24, was admitted to the Barnes Hospital Dec. 26, 1939. He had suffered from double vision for six months, and recently this had been accompanied by headaches, nausea and vomiting, and generalized weakness.

From the Departments of Pathology (Dr. Russell) and Neurosurgery (Dr. Sachs), Washington University School of Medicine.

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Examination disclosed diplopia, with the left eye turning in slightly, limitation of upward gaze and bilateral papilledema. A ventriculogram obtained Jan. 2, 1940 revealed that the lateral ventricles were moderately dilated. It was felt that the patient had a tumor in the posterior fossa, and cerebellar craniotomy was done. Because of the poor condition of the patient, the operation was terminated before the fourth ventricle could be explored. Following the operation, his condition became progressively worse, and he died January 3.

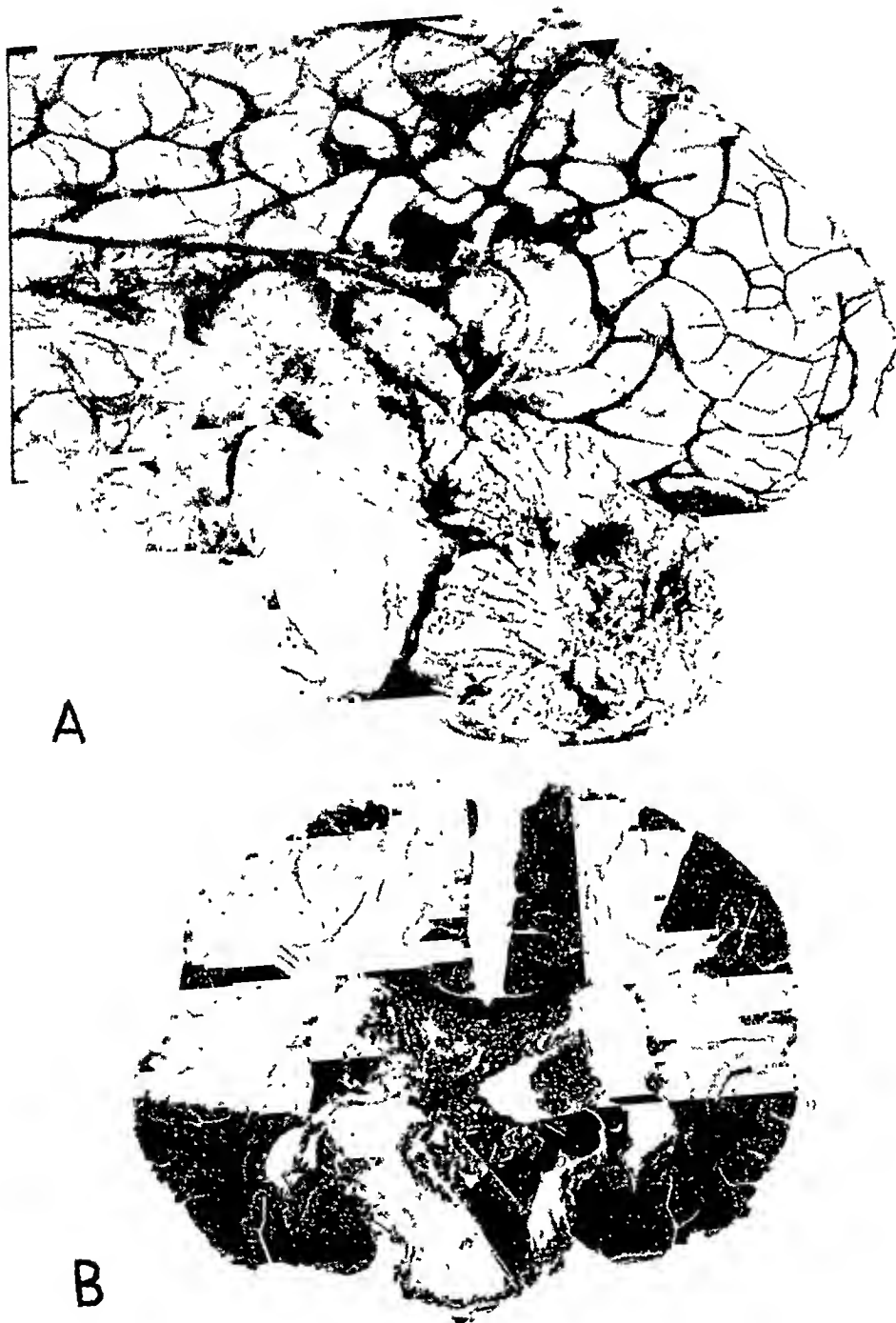


Fig. 1.—Gross appearance of pinealoma in 2 cases: *A* (case 1), sagittal section through the brain. The tumor mass is seen beneath the splenium of the corpus callosum lying on the corpora quadrigemina. It is sharply demarcated from the adjacent brain tissue. *B* (case 2), coronal section taken through the pons and the pulvinar of the thalamus. The tumor completely fills the space beneath the corpus callosum and the interbrain structures. Note the tumor tissue lining both lateral ventricles.

In reviewing the ventriculograms it is obvious that there was no air in the posterior part of the third ventricle, and the correct localization should have been made, and the pineal region should have been directly attacked via a cerebral flap.

*Necropsy* (seven hours after death; restricted to the examination of the head).—The body was well developed and well nourished. The head had been recently shaved, and there was a fresh craniotomy wound. The brain weighed 1,370 Gm. and was removed without difficulty. There was a small amount of subarachnoidal hemorrhage over the cerebellum. A sagittal section through the brain revealed a mass, 1.2 by 2.3 by 1.8 cm., attached to the habenular commissure in the roof of the third ventricle beneath the splenium of the corpus callosum. The tumor was encapsulated and projected posteriorly over the corpora quadrigemina (fig. 1 A). A moderate degree of internal hydrocephalus was present.

*Histologic Examination*.—A section taken from the tumor revealed a moderately cellular tissue composed of large cells with intermingled small cells showing no characteristic arrangement. A moderately abundant connective tissue stroma was scattered diffusely throughout the tumor. The large cells showed marked pleomorphism and no consistently characteristic shape. Some were nearly round with scant cytoplasm and measured up to 30 microns in diameter, while others were elongated, with abundant cytoplasm. The nuclei of the large cells were usually round or vesiculated with a prominent nucleolus and the chromatin heavily concentrated at the nuclear membrane. Mitotic figures were occasionally observed in the large cells. The small cells were indistinguishable from lymphocytes. They showed moderate pleomorphism and varied in size from one closely resembling a large lymphocyte with a moderate amount of basophilic staining cytoplasm and a nucleus with reticulated chromatin to one resembling a small lymphocyte with scant cytoplasm and a deeply chromatic nucleus. No mitotic figures were observed in the small cells. A phosphotungstic acid-hematoxylin stain revealed a moderate number of deep blue-staining intercellular fibrils between the large cells, some coarse and some fine (fig. 2 C).

CASE 2.—W. R., a white boy aged 17, entered the Barnes Hospital June 17, 1923. He had suffered from a cerebrospinal rhinorrhea for several months following an operation for sinusitis, which was thought to be the cause of his severe headaches. On entry he showed the signs and symptoms of meningitis, and in spite of treatment he died of meningitis ten days later. Craniotomy was not performed since the cause of his headaches was not suspected.

*Necropsy* (two hours after death; significant changes occurred only in the brain).—The convolutions were flattened, the sulci were obliterated, and the subarachnoidal space was filled with a heavy, grayish yellow exudate. A finely granular, grayish pink tumor filled the space beneath the splenium of the corpus callosum and the third ventricle. The pineal body was identified in the center of this mass by small areas of calcification. The same type of tissue was observed in the third ventricle and lining the walls of both lateral ventricles (fig. 1 B). In some areas the tumor lining the ventricles measured 1 cm. in thickness. A moderate degree of internal hydrocephalus was present.

*Histologic Examination*.—All sections of the tumor showed a moderately cellular tissue, the cells of which were of two distinct types. One was a large oval or round cell that showed only slight pleomorphism. The cells of this type tended to form closely packed groups, and these groups were surrounded by cells of the second type, which was a much smaller cell resembling a lymphocyte. In a few areas, however, cells of the two types were indiscriminately intermingled. The small cells showed slight pleomorphism and were enmeshed in a moderate amount of connective tissue stroma containing blood vessels. Mitotic figures were observed frequently in the large cells but never in the small cells. With phosphotungstic acid-hematoxylin staining a few extracellular blue-staining fibrils were noted between the large cells in the areas of the tumor where cells of the two types were indiscriminately mixed.

CASE 3.—F. C., a white boy aged 17, was admitted to the Barnes Hospital Sept. 21, 1931. He had suffered severely from prostrating headaches for three months, and recently these had been accompanied with vomiting, staggering gait and double vision. The patient's family noticed that he had become emotionally unstable and that a masklike countenance had developed. The boy was well developed, with slow speech. There was marked inequality of the pupils, with the left larger than the right, and neither reacted to light but both responded to accommodation. Both optic disks were choked, with considerable exudate. Both sixth cranial nerves were paralyzed, and upward gaze was definitely impaired. The facial nerve on the left was paralyzed. Pathologic toe signs were present on the left.

The diagnosis seemed so obvious that cerebellar craniotomy was done September 24. The exploration disclosed no tumor or other abnormality. There was no improvement, and the neurologic signs increased in severity. Therefore, November 6, a ventriculogram was made, which showed clearly that we were dealing with a tumor in the posterior portion of the third ventricle. Occipital craniotomy was performed. This time 2 Gm. of soft, grayish red tumor

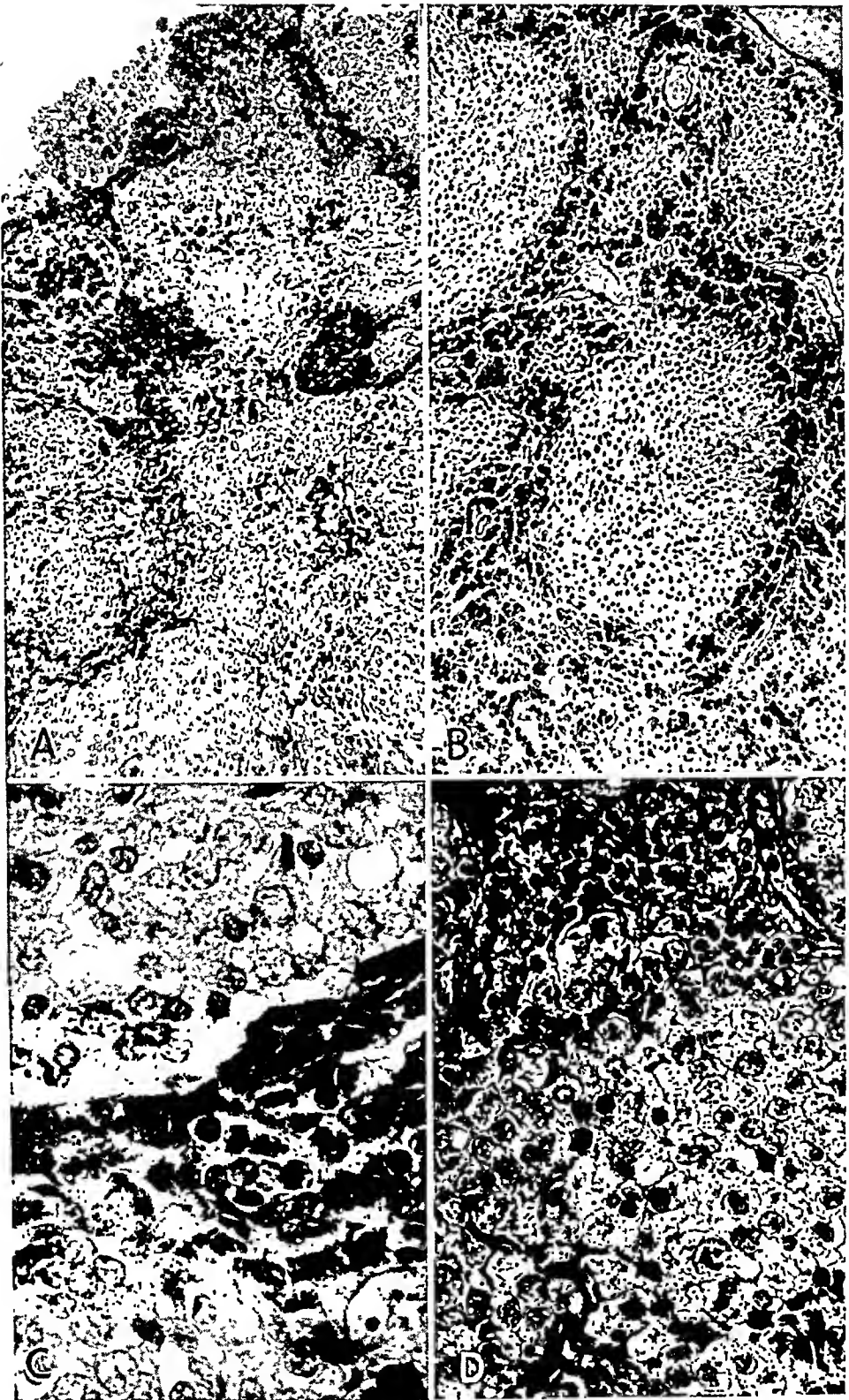


Figure 2

(See legend on opposite page)

tissue was removed from the region of the pineal body. The patient never regained consciousness and died six hours after the operation.

*Necropsy* (twelve hours after death; only pertinent observations are noted).—The brain was removed with some difficulty due to the adhesions in the posterior fossa from the previous operation. A soft, light pinkish gray tumor tissue filled the space between the splenium of the corpus callosum and the corpora quadrigemina. The tumor infiltrated the superior and inferior colliculi and the brachium conjunctivum. The lateral ventricles were moderately dilated and contained several clots of blood.

*Histologic Examination*.—All sections of tumor removed at operation and at necropsy disclosed a moderately cellular tissue, the cells of which were of two types. One type was a large cell, which showed a moderate amount of pleomorphism. In some instances it was irregularly outlined and had abundant, moderately basophilic cytoplasm with a round or slightly indented chromatic nucleus. In other instances, however, it was almost round or polygonal, with cytoplasm taking a less basophilic stain. The second type was a small cell showing moderate pleomorphism and resembling a lymphocyte, with scant basophilic cytoplasm and a deeply chromatic nucleus. In some instances it resembled a small lymphocyte, while in others it resembled a large lymphocyte. Generally the large and the small cells were indiscriminately intermingled, but in a few areas of the tumor small collections of from ten to twenty-five large cells were surrounded with broad zones of the small cells, the arrangement suggesting a mosaic pattern. Connective tissue stroma in moderate amount with small blood vessels was diffusely scattered throughout the tumor. Mitotic figures were observed frequently in the large cells but never in the small cells. A phosphotungstic acid-hematoxylin stain revealed a moderate number of deep blue-staining intercellular fibrils between the large cells.

CASE 4.—W. H., a white boy aged 14, was admitted to the St. Louis Children's Hospital Sept. 3, 1929. Beginning five months before entry he had complained of severe headaches, which had later been associated with vomiting, convulsions and periods of profound stupor. The boy was stuporous and responded poorly. The physical, sexual and mental development was in no way unusual. There was horizontal nystagmus to the right, with conjugate deviation of the eyes to the left and choking of the disks. He had several convulsions after entry, and a ventriculogram indicated a tumor in the region of the third ventricle. Transcallosal cerebral craniotomy disclosed a vascular tumor in the region of the pineal body, and 3 Gm. of tumor tissue was removed. The patient died shortly after the operation.

*Necropsy* (five hours after death, limited to the head).—The brain was easily removed, and there was a moderate amount of subarachnoidal hemorrhage over both occipital lobes and the cerebellum. The corpus callosum, the vermis and the corpora quadrigemina showed several irregularly outlined operative defects containing small amounts of clotted blood. No tumor tissue was identified, and several microscopic sections taken from these areas subsequently showed no tumor. The tumor had been completely removed at operation. There was an advanced degree of internal hydrocephalus.

*Histologic Examination*.—Several sections of the tumor removed at operation disclosed highly cellular tissue, the cells of which were of two types. One type was a large round or polygonal cell showing no pleomorphism, with clear, slightly reticulated cytoplasm that in many instances formed a clear halo around the nucleus. The nucleus of the large cell was round and moderately chromatic, and usually contained a prominent nucleolus. In some areas of the tumor only the large cells were observed, and in these areas there was only a minimal amount of connective tissue stroma with small blood vessels. In other parts of the

## EXPLANATION OF FIGURE 2

Microscopic details of pinealoma are shown.

A (case 5), photomicrograph taken from an area showing only the large cells. These cells resemble the type cell seen in oligodendroglioma. They are round or polygonal and have clear cytoplasm giving a clear halo around the nucleus. Note a prominent nucleolus in the nucleus of the cells. Hematoxylin and eosin stain;  $\times 570$ .

B (case 5), section of the tumor showing mostly small cells. One large cell is seen in the center of the field. The small cells vary in size from a cell resembling a small lymphocyte to a cell resembling a large lymphocyte. Hematoxylin and eosin stain;  $\times 1,100$ .

C (case 1), small and large pineal cells indiscriminately mixed. Coarse and fine intercellular fibrils are seen in all parts of the field. Phosphotungstic acid-hematoxylin stain:  $\times 570$ .

D (case 5), blepharoplast granules in the cytoplasm of the large cells. They are seen as small black dots. These granules are stained blue with phosphotungstic acid-hematoxylin stain. Phosphotungstic acid-hematoxylin stain;  $\times 900$ .

tumor, however, the second type of cell was seen as a small round cell resembling a small or a large lymphocyte. In some areas only the small cells were noted, but in other areas they surrounded collections of the large cells to form a mosaic pattern. The small cells were always accompanied by a moderately abundant connective tissue stroma containing blood vessels. Numerous mitotic figures were noted in the large cells but never in the small cells. A phosphotungstic acid-hematoxylin stain revealed scattered blue-staining blepharoplastic granules in the cytoplasm of the large cells.

CASE 5.—E. M., a white girl aged 10, was admitted to the St. Louis Children's Hospital Dec. 17, 1929. For five months before entry her vision had been only perception of light, and for two months she had suffered from periodic headaches, many of which were accompanied by nausea and vomiting. The child's parents had noticed that she drank unusually large amounts of water and urinated frequently. The general physical examination revealed no abnormality, and the child's physical, mental and sexual development was regarded as normal. Neurologic examination disclosed bilateral optic atrophy, marked weakness of the left internal rectus muscle, diminution of the activity of the deep reflexes, with both ankle jerks absent, and bilateral pathologic toe signs.

Because of the optic atrophy and roentgen studies of the skull which showed slight enlargement of the sella turcica, a pituitary tumor was suspected. Frontal craniotomy was performed December 23. A ventricular puncture disclosed cerebrospinal fluid under increased pressure and a moderate degree of internal hydrocephalus. A solid tumor was seen in the region of the optic chiasm; because of its close proximity to the carotid artery and the optic chiasm, it was only partially removed. The patient had an uneventful convalescence and was improved when discharged from the hospital Jan. 9, 1930.

She was readmitted to the hospital June 3. The history obtained from the parents at that time disclosed that she had recovered a moderate amount of vision following the first operation but that during the three weeks before entry she complained of painful urination and generalized weakness of the legs. The weakness of her legs became so profound that she was confined to bed for the two weeks prior to admission. There was spastic paraplegia with a definite sensory level corresponding to the ninth dorsal segment. The knee and ankle jerks were hyperactive, and there were bilateral pathologic toe signs. June 6 laminectomy was performed and soft reddish tissue removed from around the spinal cord at about the fifth dorsal segment. Convalescence was satisfactory, but there was only slight return of sensation in the lower extremities with no return of motor power. High voltage roentgen radiation was given over the lesion. The patient was discharged from the hospital unimproved July 3.

Specific details of the patient's course after her discharge from the hospital are not known except that she died from her disease in another hospital several months later.

*Histologic Examination.*—Sections from the tumor removed at the first and at the second operation all revealed the same type of growth. The tumor was composed of masses of large round or polyhedral cells intermingled with cells of a second type resembling lymphocytes. In some instances the small cells surrounded large groups of closely packed large cells, forming a mosaic pattern. The large cells showed no pleomorphism and had a moderate amount of slightly reticulated clear cytoplasm that in many instances gave a clear halo surrounding the round chromatic nucleus. In many areas only the large cells were found (fig. 2A), and the diagnosis of oligodendroglioma would have been strongly considered had only these areas been examined, for these large cells strikingly resembled the type cell of oligodendroglioma. The finding of small cells in other parts of the section and a mosaic pattern (fig. 3D) plus the fact that the large pineal cells frequently contained blepharoplastic granules (fig. 2D), which are never seen in oligodendroglial cells, were important differential diagnostic points. The small cells resembled lymphocytes and showed gradations in size from a form resembling a large lymphocyte to a form resembling a small lymphocyte (fig. 2B). Mitotic figures were frequently observed in the large cells but were never observed in the small ones. A phosphotungstic acid-hematoxylin stain of the tumor disclosed a moderate number of blue-staining blepharoplastic granules within the large cells.

CASE 6.—R. T., a Negro boy aged 9, was admitted to the St. Louis Children's Hospital Dec. 5, 1939. He had suffered from attacks of severe headache for four months and had vomited severely on several occasions. He was well developed, alert and cooperative, with no abnormal physical, mental or sexual development. The significant observations were bilateral unsustained ankle clonus, dilated pupils, choked disks and slight bilateral exophthalmos. December 12 a ventriculogram was obtained which was interpreted as indicating a lesion of the posterior fossa. Cerebellar craniotomy was performed, but no tumor was found. In reviewing the plates now it is clearly evident that there was a lesion in the posterior part of the third ventricle, as no air filled this region. Cerebellar craniotomy was performed

again December 29, because the patient's condition had become progressively worse. At this time 14 Gm. of tumor tissue lying anterior to the cerebellum in the region of the corpora quadrigemina was removed by sucker. Following the operation, the patient had marked respiratory difficulty and was kept in an oxygen tent for several weeks but died Feb. 21, 1940.

*Necropsy* (twenty hours post mortem).—Except for advanced bronchopneumonia in the lower lobes of the lungs the significant observations were in the brain. This was removed with considerable difficulty due to fibrous adhesions from the two operations. The weight of the brain was normal, 1,100 Gm. Operative defects were observed on the vermis, the colliculi and the floor of the fourth ventricle. A small piece of grayish pink tissue, measuring 1.5 by 1.3 by 0.8 cm., was attached to the tentorium. The third and the lateral ventricles were moderately dilated.

*Histologic Examination.*—Sections of the tumor removed at operation and the nodule of tumor attached to the tentorium at necropsy all disclosed a highly cellular tissue, the cells of which were of two types. One type was a large round or polygonal cell that had abundant clear cytoplasm and a round, moderately chromatic nucleus. The cells of this type showed little or no pleomorphism and were closely packed into large groups which in turn were

TABLE 1.—Summary of the Pertinent Pathologic Observations in the Seven Cases Reported

No.	Age, Yr.	Sex	Location of Tumor	Hydrocephalus	Large Cells				Small Cells		
					Pleo-morph-ism	Mitotic Figures	Bleph-aro-plasts	Inter-cellular Fibrils	Pleo-morph-ism	Mitotic Figures	Mosaic Pattern
1	24	M	Pineal region	Moderate	+++	+	0	++	++	0	0
2	17	M	Pineal region, corpora quadrigemina, lateral ventricle	Moderate	+	++	0	+	+	0	++
3	17	M	Pineal region, corpora quadrigemina	Moderate	++	+++	0	+	++	0	+
4	14	M	Pineal region	Advanced	0	+-+	+	0	++	0	++
5	10	F	Pineal region	Moderate (determined at operation)	0	++	++	0	++	0	+
6	9	M	Pineal region, extension to tentorium	Moderate	0	+++	+++	0	++	0	++
7	2	F	Pineal region	Advanced (determined at operation)	0	0	0	0	+	0	++++

surrounded by cells of the second type, this arrangement giving the effect of a mosaic pattern. The second type of cell was a small round cell resembling a lymphocyte with a small amount of basophilic cytoplasm and a round, slightly oval or indented, deeply chromatic nucleus. The small cells showed variations in size and shape from cells closely resembling large lymphocytes to cells resembling small lymphocytes. Mitotic figures were frequently observed in the large cells but never in the small cells. Many blue-staining blepharoplastic granules were seen within the cytoplasm of the large cells in a phosphotungstic acid-hematoxylin stain.

CASE 7.—C. S., a white girl aged 2, was admitted to the St. Louis Children's Hospital May 24, 1927. For two months before entry her parents had noticed that she frequently stumbled and occasionally fell. During this time there had been periodic attacks of vomiting. There was no abnormal physical, mental or sexual development. Neurologic examination revealed marked incoordination of the movements of the extremities, bilateral pathologic toe signs, bilateral sustained ankle clonus, bilateral papilledema and dilatation of pupils, which did not react to light. May 27 cerebellar craniotomy disclosed that the lateral ventricles were markedly dilated, and 12 Gm. of grayish pink tumor tissue was removed from the region of the pineal body by splitting the vermis. The patient made an uneventful recovery and was discharged from the hospital July 17 in good condition. Further attempts to observe the patient were unsuccessful, and it is not known whether the operation effected a complete cure or not.



*Histologic Examination.*—Sections of the tumor disclosed tissue composed of cells of two types. One type was a large cell having an ill defined cell membrane with clear cytoplasm and a small hyperchromatic nucleus. These cells were closely packed into large masses but showed no appreciable pleomorphism. The second type of cell was a small cell resembling a large lymphocyte. The cytoplasm was basophilic, and the nucleus contained abundant deeply basophilic chromatin that in some instances was slightly reticulated. These cells were grouped together in broad zones and surrounded collections of the large cells. This unusual arrangement of the two types of cells gave to the section when examined under low power magnification the appearance of a mosaic pattern (fig. 3B). A phosphotungstic acid-hematoxylin stain of the tumor revealed no intercellular or intracellular fibrils or blepharoplastic granules. There was discernible in this stain, however, a connective tissue stroma accompanying the collections of the small cells, with a moderate number of small blood vessels. No connective tissue stroma or blood vessels were observed in the collections of the large cells.

The pertinent gross and histologic observations made in the 7 cases are summarized in table 1.

#### HISTOGENESIS OF THE TWO TYPES OF CELLS IN PINEALOMA

Our knowledge of the histogenesis of the two types of cells observed in pinealoma is derived from studies of the development of the pineal body in man. The anlage for the pineal body appears in man during the second month of fetal life with hyperplasia of the ependymal cells in the posterior part of the roof of the diencephalon. Coincident with the hyperplasia and the initial piling up of the primitive ependymal cells there appears a small evagination of the wall of the third ventricle into the cell mass to form a small diverticulum. According to Krabbe,<sup>7</sup> this diverticulum, by splitting the cell mass into an anterior and a posterior part, presumably forms two separate anlagen, the anterior one representing the parapineal organ and the posterior one the pineal organ.

In the fourth month of fetal life the anterior and posterior anlagen increase in size, with the result that there is marked narrowing of the cavity of the pineal diverticulum. Fusion of the anterior and posterior anlagen with obliteration of the diverticulum from the third ventricle is effected in the fifth prenatal month. As a result of the fusion of the two anlagen the pineal body assumes the conical form which characterizes the fully developed stage.

During the sixth month of fetal life there is marked increase in the bulk of the organ. At about the middle of the sixth fetal month large masses of small, deeply staining cells appear throughout the developing organ. With further development these small cells show a characteristic arrangement into cordlike masses, which tend to surround collections of large, less deeply staining cells. This arrangement, the collections of large clear cells (parenchymal cells) surrounded by dense masses of small, deeply staining cells, gives the tissue an appearance strikingly unusual, which has been likened to a mosaic pattern (fig. 3 A and C).

The further development of the pineal body until birth consists of an increase of the vascularity of the stroma, which is most abundant in the areas containing the small cells, and a moderate increase in the size of the organ, with the collections of large cells becoming relatively more prominent. During the first postnatal month there begins progressive diminution in the number of the small dark-staining cells. By the end of the ninth postnatal month virtually all the small cells have disappeared and there is a marked increase in fibrous tissue in the areas previously occupied by these cells. There is little change in the histologic structure of the pineal body with advancing years. The islands of parenchymal cells remain, the septums of connective tissue surrounding the large cells increase slightly, and focal areas of calcification begin to appear throughout the organ about the second year of life. For a more detailed and complete description of the histogenesis and

7. Krabbe, K. H.: *Anat. Hefte* 54:191, 1916.

development of the pineal body the reader is referred to the splendid studies of Krabbe,<sup>7</sup> Marburg<sup>8</sup> and Globus and Silbert.<sup>4</sup>

The large cell is frequently referred to as the "parenchymal cell" of the pineal body (del Río Hortega<sup>9</sup>; Globus and Silbert<sup>4</sup>; Horrax and Bailey<sup>3</sup>), and it is universally agreed that it develops from the pineal anlage and is of nervous origin. Hortega<sup>9</sup> demonstrated in histologic studies that this cell has an unusual type of process with a bulbous ending. Russell and Gregory<sup>10</sup> concluded from comparative histologic studies that it should be regarded as an altered visual cell from the retina of the old pineal eye. Their conclusion is based on the finding of similarly shaped processes on the visual cells from paired eyes of certain vertebrate forms in which the paired eyes have undergone retrogressive evolutionary changes.

The histogenesis of the small cell is disputed. Horrax and Bailey<sup>3</sup> regarded the small cell as a glial cell of neuroectodermal origin which developed with the parenchymal cell in the pineal anlage. Globus and Silbert,<sup>4</sup> on the other hand, after a careful study of the histogenesis of the human pineal body concluded that the small cells were derived from mesenchyme and were capable of differentiating into fibroblasts. These investigators observed that the marginal grouping of the small cells around the large collections of parenchymal cells making up the basic mosaic pattern of the pineal body in the infantile stage disappeared in the maturing organ, the small cells being apparently replaced by fibrous tissue. The disappearance of the small cells was interpreted by them as representing a transformation of the small cells into fibroblasts.

The hypothesis of Globus and Silbert<sup>4</sup> of a mesenchymal origin for the small cells is open to question, since the presence of large collections of mesenchymal cells in the pineal anlage was unexplained by these authors and no migration of mesenchymal cells into the pineal anlage was described in their study of the histogenesis of this structure. Moreover, the transformation of the small cells into fibroblasts in the maturing organ as suggested by them is certainly not the most logical explanation, for with the degeneration and disappearance of the small cells in the adult stage an expected sequence of events would probably be an increase in fibrous tissue. The concept of Horrax and Bailey<sup>3</sup> of a neuroectodermal origin for the small supporting cells that develop from the pineal anlage is more reasonable.

Because the tumors classified as pinealoma characteristically contain the two types of cells observed in pineal tissue at the time of birth, it is concluded that their cells are likewise of neuroectodermal origin. Pinealoma, therefore, is merely another type of glioma representing neoplastic pineal tissue at its highest stage of development, namely, at about the time of birth.

#### CRITERIA FOR THE DIAGNOSIS OF PINEALOMA

Considerable difference of opinion prevails concerning just which type of primary pineal tumor should be called pinealoma. Horrax and Bailey<sup>3</sup> described a type of pinealoma which was named "spongioblastic pinealoma." They regarded spongioblastic pinealoma as a type of poorly differentiated glial tumor of the pineal anlage occurring before the two types of cells were differentiated. It was admitted, however, that in many instances it was impossible to differentiate spongioblastic pinealoma from glial tumors primary in other parts of the brain. Globus and Silbert,<sup>4</sup> on the other hand, did not recognize even the possibility of such a type of glial tumor arising in the pineal body because they were unable to demonstrate

8. Marburg, O.: *Arch. a. d. neurol. Inst. a. d. Wien. Univ.* **17**:217, 1908.

9. del Río Hortega, P.: *Arch. de neurobiol.* **3**:359, 1922.

10. Russell, W. O., and Gregory, W. K.: Unpublished data.



any glial cells in the pineal body in their study of the histogenesis of that organ. In the 7 cases of pineal tumor reported by them the diagnosis of pinealoma was given in every instance. As for those pineal tumors not showing two types of cells or a mosaic pattern, they interpreted those as representing some phase in the differentiation of the cells in the pineal anlage before that showing the two characteristic cells.

Still a third type of supposedly characteristic primary pineal tumor was reported by Baggenstoss and Love.<sup>6</sup> In reporting 10 cases of pineal tumor these authors recognized pinealoma with its two types of cells, the spongioblastic pinealoma of Horrax and Bailey<sup>3</sup> and, in addition, a third type, called by them pineal ependymoma. As can be judged from the description and photomicrographs, this type possessed nothing which would distinguish it from other tumors termed ependymoma.

The most satisfactory classification for any tumor is one based on the cell from which the tumor is believed to originate, as advocated by Mallory<sup>11</sup> and by Ewing.<sup>12</sup> A nomenclature depending on purely descriptive, morphologic criteria or on what the tumor may produce, exemplified by such terms as "round cell sarcoma" and "cholesteatoma," is often misleading and in the opinion of many oncologists not strictly scientific. For this reason we have adopted a rigid concept of what type of tumor is deserving of the name "pinealoma." The designation of a tumor as spongioblastic pinealoma or pineal ependymoma appears to us not justified if there is no definitely distinguishing characteristic feature of the tumor to differentiate it from a glial tumor arising in some other area of the brain. Just because a tumor is situated in the pineal region does not warrant calling it pinealoma. This error occurs frequently in the literature. To illustrate this point, we have recently observed a primary tumor of the pineal body that histologically was typical spongioblastoma multiforme. According to the classification of Horrax and Bailey,<sup>3</sup> the diagnosis for this tumor would be spongioblastic pinealoma. We feel, however, that this tumor is more correctly called spongioblastoma multiforme of the pineal body, for its histologic characteristics were no different from those of spongioblastoma multiforme arising in another part of the brain.

We have been unable to confirm the conclusions of Globus and Silbert<sup>4</sup> that primary tumors of the pineal body not showing two types of cells represent differential stages in the developing pineal body before the appearance of the two types of cells. The possibility is freely admitted, however, that a tumor may arise from pineal tissue which is not sufficiently differentiated to show the two characteristic types of cells. Because the pineal anlage is derived from nerve tissue, such a tumor should exhibit a type of growth resembling some type of glioma. We have observed 4 cases of primary tumor of the pineal body that did not fulfil our criteria of two types of cells, and in each instance it was possible to classify the tumor as some type of glioma. For example, in case 10, reported by Globus<sup>13</sup> as a case of pinealoma, the histologic description and the photomicrograph suggest astrocytoma, and the author described "well differentiated astrocytes."

Since pineal tumors showing two types of cells and a mosaic pattern reproduce pineal tissue in its most highly developed form, we believe that the term "pinealoma" is correctly applied only if it is used exclusively for those pineal tumors containing the two characteristic types of cells seen in normal pineal tissue at the time of birth. The presence of a mosaic pattern is not regarded as necessary for

11. Mallory, F. B.: *J. M. Research* **13**:113, 1905.

12. Ewing, J.: *Neoplastic Diseases*, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 9-13.

13. Globus, J. H.: *Arch. Path.* **31**:533, 1941.

the diagnosis, because occasionally pineal tumors showing the two types of pineal cells will show no mosaic arrangement. The term "pinealoma" as used in this study is based on this concept of the tumor.

#### LITERATURE AND PREVIOUSLY REPORTED CASES OF PINEALOMA

A review of the literature of pineal tumors reveals 51 cases that in our opinion are verified instances of pinealoma. Our criterion that this type of tumor must have two types of cells was rigidly followed, and in all the cases accepted a photomicrograph or a histologic description verified the presence of the two characteristic cells. No doubt, several more cases of pinealoma have been reported that are not included here, for in many instances the histologic description of the tumor was inadequate to establish the diagnosis by our criteria, and in other instances a histologic description was not given. Baggenstoss and Love<sup>6</sup> reported 10 cases of pineal tumor but gave a histologic description of the tumor in only 4. Horrax<sup>14</sup> reported a case in which he gave pinealoma as the pathologic diagnosis but did not give a histologic description. We were unable to obtain the original publications of Hempel<sup>15</sup> and Steiner and Johan.<sup>16</sup> The collected cases, including the 7 reported in this paper, are listed in table 2.

#### STUDY OF THE COLLECTED CASES

The general features of pinealoma can be determined by an analysis of this group of 58 cases (tables 1 and 2) under the following heads:

*Age and Sex.*—As judged from the cases collected for this study, pinealoma occurs most frequently in young adults between the ages of 15 and 25 years (28 cases). It is seen much less frequently in persons at or under 15 years (17 cases) and beyond the age of 25 years (13 cases). It occurs preponderantly in males (88 per cent); only 5 of the 58 tumors collected occurred in females.

*Symptoms.*—The general signs of increased intracranial pressure were present in nearly all instances. Headache was the first symptom noted by 41 of the 58 patients and was associated with vomiting in 18. Other symptoms, such as polyuria or a symptom referable to one or more cranial nerves without headache and vomiting, were the first symptoms complained of by only 13 patients. Paralysis of one or more cranial nerves was observed in 36 patients. Disturbance of vision or papilledema was the most constantly observed neurologic sign, for such disturbances were steadily absent in only 6 of the cases. Most interesting is the observation that precocious puberty was present in only 3 of the 17 patients at or below the age of 15 years. Diabetes insipidus was observed in 15 of the patients, and other symptoms indicating some endocrine imbalance, such as obesity or hypogonadism, were noted in 10. Loss of the ability to look upward was specifically mentioned in 21 cases, not mentioned in 29 and definitely stated to be not present in only 8 instances. This symptom is indicative of a lesion in the posterior part of the third ventricle and frequently is noted with pineal tumors.

*Rate of Growth.*—As can be judged from the duration of the patients' symptoms before seeking medical aid, the tumors classified as pinealoma are fairly rapidly growing ones, for 32 patients had symptoms that could be attributed to intracranial disease for less than a year, and 20 of these, for less than six months. In 22 patients the symptoms were noted for over a year, the longest period being

14. Horrax, G.: Arch. Neurol. & Psychiat. 37:385, 1937.

15. Hempel, H. K.: Ein Beitrag zur Pathologie der Glandula Pinealis, Inaug. Dissert., Leipzig, B. Georgi, 1901.

16. Steiner and Johan: Orvosi hetil. 66:367, 1922.

TABLE 2.—Reported Cases of Pinealoma

Number	Author	Age of Patient	Initial Symptoms			Important Neurologic Signs			Endocrine Disturbances			Duration of Symptom before Treatment	Treatment			Location			Autopsy	Mosaic Pattern	Final Result
			Headache	Vomiting	Others	Cranial Nerve	Loss of Upward Gaze	Visual Disturbances and Papilledema	Precocious Puberty	Diabetes Insipidus	Obesity or Glandular Disturbances		Operation	Irradiation	Pineal Region 3d Ventricle	Lateral Ventricle	Subarachnoid Space				
1	Reinhold, H.: Deutsches Arch. f. klin. med. <b>39</b> : 1, 1886.	19	M	+	0	+	+	+	+	0	0	0	7 mo.	0	0	+	0	0	+	0	Death
2	Howell, C. M. H.: Proc. Roy. Soc. Med. <b>3</b> : 65, 1910																				
3	Case 1	42	M	+	0	+	+	+	+	+	0	0	6 mo.	+	?	0	+	0	+	?	Death
4	Case 2	22	M	0	+	+	+	+	+	+	0	0	3 mo.	+	?	0	+	0	+	?	Death
5	Case 3	20	M	+	0	0	?	+	+	+	0	0	5 mo.	+	+	0	+	0	+	?	Death
6	Rorschach, H.: Beitr. z. klin. Chir. <b>83</b> : 431, 1913.	27	M	0	0	0	?	+	+	+	0	0	2 yr.	+	?	0	+	+	+	?	Death
7	Uemura, S.: Frankfurt. Ztschr. f. Path. <b>20</b> : 380, 1917.	59	M	?	?	?	?	?	?	?	0	?	?	?	?	?	+	0	+	?	Death
8	Skogg, A. L.: New York M. J. <b>107</b> : 1199, 1918.	9	M	+	+	0	0	?	+	+	+	0	+	4 mo.	0	0	+	0	+	?	Death
9	Löwenthal, K.: Beitr. z. path. Anat. u. z. allg. Path. <b>67</b> : 207, 1920	23	M	+	0	0	+	?	+	+	0	+	+	4 mo.	0	0	+	0	+	+	Death
10	Perbinger, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. <b>95</b> : 741, 1925	33	M	+	0	+	+	?	+	+	0	0	1 yr.	0	0	+	0	+	+	+	Death
11	Horrox and Bailey <sup>2</sup> (1925)																				
12	Case 4	12	M	+	+	0	+	?	+	+	+	0	3 mo.	0	0	+	0	0	+	+	Death
13	Case 7	23	M	+	0	+	+	?	+	+	0	0	1 yr.	+	0	+	0	0	+	+	Death
14	Case 8	13	M	+	+	+	?	+	+	+	0	0	3 yr.	+	0	+	0	0	+	+	Death
15	Case 9	17	M	+	+	0	+	?	+	+	0	0	1 yr.	0	0	+	0	0	+	+	Death
16	Case 10	20	M	+	+	0	+	+	+	+	0	+	9 mo.	+	0	+	0	0	+	+	Death
17	Badado, M.: Arch. argent. de neurol. <b>1</b> : 10, 1927																				
18	Case 1	17	M	+	0	0	+	+	+	+	0	0	3 mo.	+	0	+	0	0	+	+	Death
19	Case 2	15	M	0	0	+	+	+	+	+	0	+	1 yr.	0	0	+	+	0	+	+	Death
20	Arend, R., and Schusterówna, H.: Polska gaz. lek. <b>9</b> : 381, 1930	10	M	+	0	0	+	?	+	+	0	+	2 yr.	0	0	+	0	0	+	?	Death
21	Fulton, J. F., and Bailey, P.: J. Nerv. & Ment. Dis. <b>69</b> : 1, 1929 (case 2)	28	F	0	0	+	0	0	+	0	0	+	5 yr.	+	0	?	+	0	+	?	Death
22	Liebert: Deutsche Ztschr. f. Nervenhe. <b>108</b> : 101, 1929.	10	M	+	+	0	+	+	0	0	0	0	6 mo.	0	0	+	0	0	+	+	Death
23	Kux, E.: Beitr. z. path. Anat. u. z. allg. Path. <b>87</b> : 59, 1931	22	M	+	+	+	+	?	+	+	0	0	6 mo.	0	0	+	0	0	+	0	Death
24	Harris and Cairns (1932) <sup>10</sup>	20	M	+	0	0	?	0	+	+	0	0	2 yr.	+	+	+	?	?	0	+	Indeterminate, well after 2 yr.
25	Globus (1932) <sup>17</sup>	2	M	0	0	+	+	?	+	+	0	0	2 mo.	+	+	+	+	0	+	0	Death
26	Guillain, G.: Vol. jubilaire, Marinisco, 1933, p. 281.	25	M	+	+	0	+	+	+	0	0	0	2 yr.	0	0	+	0	0	+	0	Death
27	Vincent, C., and Rappoport: Rev. neurol. <b>1</b> : 517, 1933.	40	M	0	0	+	+	?	+	+	0	0	2 yr.	+	0	+	0	0	+	?	Death
28	Stringer, S. W.: Yale J. Biol. & Med. <b>6</b> : 375, 1934.	27	M	0	0	0	0	0	0	0	0	0	6 mo.	0	0	+	0	0	+	+	Death



six years (Dandy,<sup>5</sup> case 3). Apparently there is no correlation between the duration of the disease before the patient comes to the hospital and the type of growth and differentiation of the tumor, for in case 7 (table 1) of our series the total duration of the symptoms was only two months and the tumor showed the most highly developed and differentiated type of growth.

*Gross Appearance and Growth Behavior.*—The gross appearance of the tumor of this group of 58 cases was extremely variable. In some instances it was a small encapsulated mass of tissue, not much larger than the pineal body itself, which remained localized and caused symptoms only by pressure on adjacent structures (fig. 1 *A*). In other instances it was disseminated throughout the lateral ventricles (18 cases) (fig. 1 *B*) and into the cerebral and spinal subarachnoidal spaces (7 cases). The usual finding, however, was a nonencapsulated infiltrating tumor in the region of the pineal body on the habenular commissure, which had extended either anteriorly into the third ventricle or posteriorly to involve the corpora quadrigemina and the interbrain structures.

*Microscopic Appearance.*—The discussion of the histologic features of pinealoma and its cellular types given in this section is based on the 7 cases of pinealoma observed by us. Only in regard to the mosaic pattern was it possible to include the results of a study of the cases collected from the literature. The histologic structure of pinealoma is extremely variable since it consists of cells of two distinctly different types each of which may show individual pleomorphism along with a quantitative difference in the ratio of the cells of one type to the cells of the other. Still another variable was the tendency of the cells in most instances to show a characteristic morphologic arrangement that has been likened to a mosaic pattern. The variations observed in each cell and the mosaic pattern are discussed separately.

The large cells (parenchymal cells) showed great variation in outline and form in different tumors and oftentimes in the same tumor, so that no one description can be given that will adequately cover the wide morphologic variation. The large cells in cases 4, 5 and 6 (table 1) were round to oval, with clear cytoplasm and a round, moderately chromatic nucleus (fig. 2 *A*). Because of the clear halo of cytoplasm, this type of cell strikingly resembled the type cell of oligodendroglioma. It showed no intracellular or extracellular fibrils when stained with phosphotungstic acid-hematoxylin; however, it frequently contained small round or slightly elongated blepharoplast granules (fig. 2 *D*). In other tumors (cases 1, 2 and 3 of table 1) the large cells were associated usually with a moderate number of deep blue-staining intercellular fibrils when stained with phosphotungstic acid-hematoxylin (fig. 2 *C*). Mitotic figures were present in moderate numbers in the large cells in 6 of the tumors, but mitotic figures were not seen in the tumor in case 7. This fact is highly significant, for this tumor was the most highly differentiated tumor of the group and more closely resembled pineal tissue as seen at the time of birth than any of the other tumors studied. Here the large cells showed still another type of variation and more closely resembled the parenchymal cells in the pineal tissue present at birth than did cells in any of the other tumors. This type of large cell contained a small round hyperchromatic nucleus with clear cytoplasm having poorly defined borders (fig. 3 *B*).

Because the large cells in pineal tissue have characteristic processes with bulbous endings which are demonstrated only with the silver impregnating technics, attempts were made to show similar processes on the large cells in pinealoma. In none of the 7 tumors studied, however, were we able to demonstrate any processes resembling those described by Hortega<sup>9</sup> for pineal cells. Processes resembling

those of normal pineal cells have been described on the large cells of pinealoma by Horrax and Bailey.<sup>3</sup> Bielschowsky's staining method for neurofibrils, Hortega's method for pineal parenchyma and Hortega's method for astrocytes were tried on each tumor without results. Characteristic processes were shown, however, on the large pineal cells in normal pineal tissue by these methods.

The small cells in most instances were indistinguishable from small or large lymphocytes. They were characteristically round and contained moderately basophilic cytoplasm with a hyperchromatic round or slightly indented nucleus. In most of the tumors the small cells showed gradations in size from a cell resembling a large lymphocyte, on the one hand, to a cell resembling a small lymphocyte, on the other (fig. 2 B). Careful study of all the tumors disclosed no mitotic figures in the small cells. Because the small cells in pinealoma so closely resemble lymphocytes the question is reasonably asked whether they are truly neoplastic cells and part of the tumor or actually lymphocytes that have infiltrated the tumor as is occasionally seen in some types of epithelial tumors. We believe that they are neoplastic cells because they resemble the small cells in infantile pineal tissue, which are admittedly not lymphocytes but a type of glial cell. Moreover, they show a characteristic arrangement in the tumor that reproduces the mosaic pattern of infantile pineal tissue, which further identifies them with pineal tissue.

The mosaic pattern, shown in 34 of the 58 tumors, in its most highly differentiated form was a complete reproduction of the cellular arrangement in pineal tissue as observed at the time of birth (fig. 3). In only 13 of the 58 tumors was there no discernible mosaic pattern. The description was not complete enough in 11 cases to allow one to be certain whether a mosaic arrangement was present or not. Those tumors showing a poorly developed mosaic pattern were regarded as examples of a more anaplastic type of tumor. The age of the patient apparently was not a determining factor in the development of the mosaic pattern, for the tumors from some of the youngest (Globus<sup>17</sup>) and oldest patients (Friedman and Plaut<sup>18</sup>) showed no mosaic pattern. The mosaic pattern can be regarded as a fairly constant feature of pinealoma since 34 of the 58 tumors showed some semblance of it. The absence of a mosaic pattern does not in any way vitiate the diagnosis of pinealoma if the tumor contains the two characteristic cells. In fact, the point should be strongly emphasized that in some instances, as was observed in cases 4 and 5 (table 1), large areas of the tumor may be composed solely of the large cells without any small cells, while in other areas of the tumor the two types of cells will form a fairly characteristic mosaic pattern.

*Treatment and Prognosis.*—Thirty-two of the 58 patients were treated by operation. High voltage roentgen radiation was used in the treatment of 4 patients, but no improvement that could be attributed to the rays was noted. Death was known to be the final result in all of the cases except 3 (cases 21, 30, 58, table 2). In case 21 (Harris and Cairns<sup>19</sup>) the patient completely recovered from the operation for removal of the tumor and was reported well, without further detail, two years after the operation. In case 30 (Dandy<sup>5</sup>) the patient recovered from the operation, but details of the patient's subsequent course are not reported. It is not known whether a permanent cure was effected in case 58, contributed by us, since the patient could not be located after she left the hospital, although she was symptom free and recovered from the operation at the time of discharge.

17. Globus, J. H., in *Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman*, New York, International Press, 1932, vol. 2, p. 491.

18. Friedman, E. D., and Plaut, A.: *Arch. Neurol. & Psychiat.* **33**:1324, 1935.

19. Harris, W., and Cairns, H.: *Lancet* **1**:3, 1942.

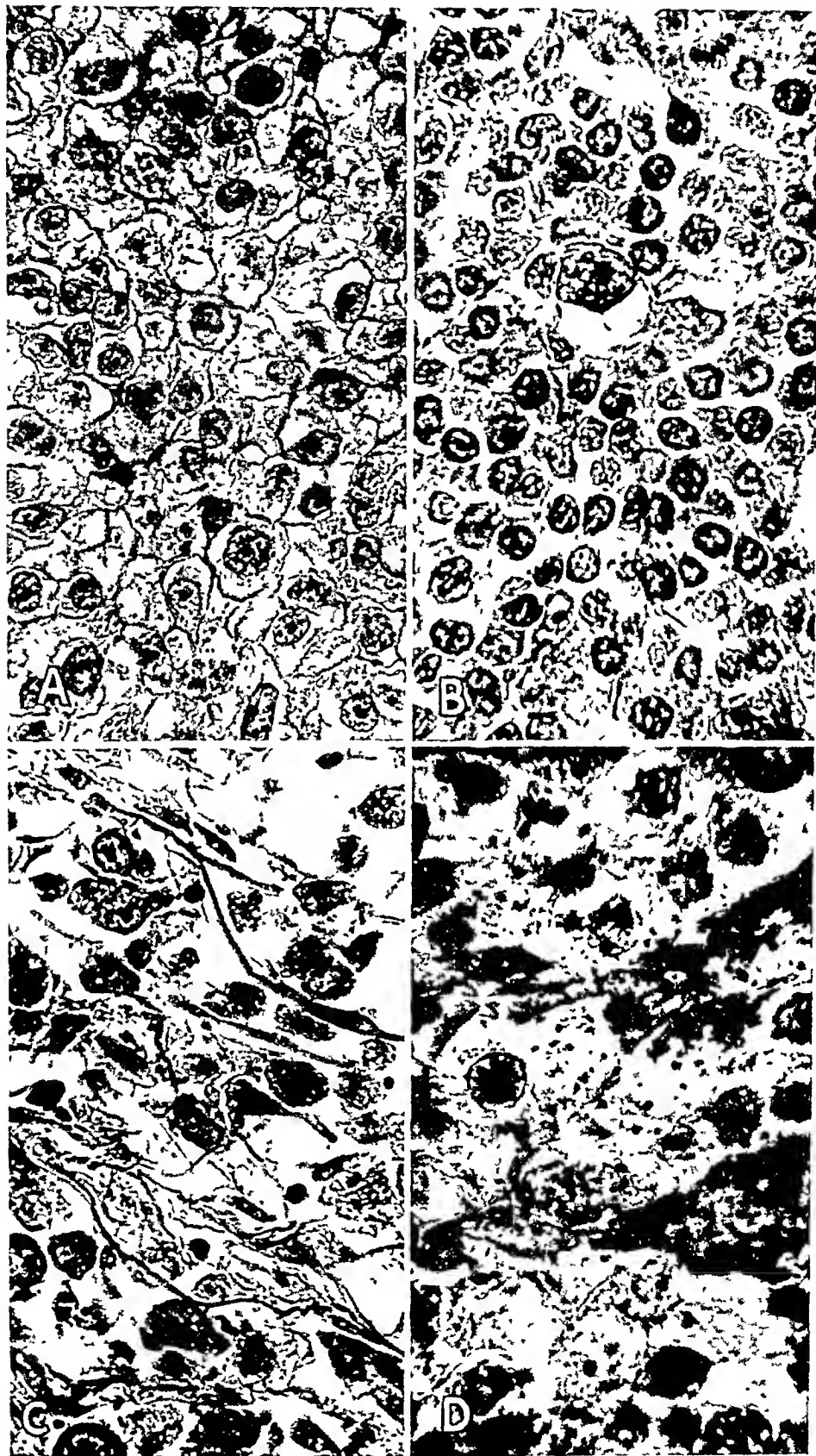


Figure 3  
*(See legend on opposite page)*



Since death was known to be the final outcome in all but 3 cases, it is clear that at present pinealoma offers an extremely poor prognosis. The location of the growth in the roof of the third ventricle is an important factor in accounting for its extremely poor prognosis, because extension of the tumor to the third ventricle or the interbrain structures is the rule, so that complete surgical removal is impossible.

We have had personal communications with German, Dandy and Horrax concerning their experiences with pinealoma. German cited a patient whom he treated by operation who is living and free from evidence of recurrence five years after the removal of the tumor. Dandy mentioned a patient who is living and well five and a half years after operation and others who have lived for two to three years following operation. Horrax reported a young woman who is well and without symptoms of disease five years after the removal of a tumor diagnosed as pinealoma and a second patient who lived for eight years after removal of a tumor with this diagnosis. In the latter's case roentgen treatment was given immediately after operation, for it was known that all the tumor was not removed. The patient improved with the roentgen therapy and was well for five years when symptoms of intracranial disease returned. She survived three more years and died following an operation for radical removal of her tumor eight years after the first operation.

#### COMMENT

In this study three points merit special comment and consideration: first, the part played by pinealoma in the production of the precocious puberty associated with pineal tumors; second, a discussion of the prevailing concept of the physiologic mechanisms involved in this syndrome, and third, the unusual type of neoplasia shown by pinealoma.

With the collection of 58 histologically verified cases of pinealoma opportunity is afforded to determine whether or not the tumor itself is directly concerned with the syndrome of precocious puberty. The implied reasoning often encountered is that the pineal body is some form of endocrine gland and neoplasia of its tissue produces a tumor which in turn elaborates a hormone that is directly responsible for the precocious sexual development. If such a hypothesis is correct, only pinealoma, representing neoplasia of pineal tissue, should be significant, since other types of tumor arising in the pineal body, such as glioma or teratoma, are known not to be associated with precocious puberty when observed in other parts of the brain (glioma) or the body (teratoma).

#### EXPLANATION OF FIGURE 3

In this figure a comparison is made between pineal tissue at the time of birth and pinealoma.

*A*, section of pineal tissue from a full term infant. Collections of the large pineal cells are surrounded by cordlike collections of the small cells. The arrangement of small and large cells gives the characteristic mosaic pattern. Hematoxylin and eosin stain;  $\times 160$ .

*B* (case 7), section of the tumor showing large and small cells forming a mosaic pattern. Note that the blood vessels visible in the section are all contained in the areas of the small cells. Compare with *A*, showing pineal tissue at the time of birth. Hematoxylin and eosin stain;  $\times 160$ .

*C*, higher magnification of a part of the section shown in *A*. The large cells are shown in the upper and lower parts of the field with a mass of small cells, deeply basophilic in staining, in the center of the section. Hematoxylin and eosin stain;  $\times 570$ .

*D* (case 5), section showing large cells in the lower right side of the field and small cells in the upper left side. Note the mitotic figure in the center of the group of the large cells in the lower right center position and the characteristic grouping of the large and the small cells. Compare with *C*. Phosphotungstic and hematoxylin stain;  $\times 510$ .



The first point to be considered is the fact that pinealoma is not a tumor of preadolescent years but has its highest incidence (48 per cent of all cases) in the first ten year period following puberty. There is no demonstrable histologic change in the pineal body occurring after puberty that could account in any way for the high incidence of pineal tumors in this period. Only 17 of the 58 patients (29 per cent of all cases) were at or below the age of puberty; so from a purely statistical standpoint there has been but a small number of tumors diagnosed as pinealoma that could have produced precocious puberty. Certainly the most remarkable finding in this study is that of the 17 patients with pinealoma at or below the age of 15 years only 3 showed associated precocious sexual development.

The observations reported by Bing, Globus and Simon<sup>2</sup> on teratoma of the pineal body are cited for comparison with those on pinealoma. In a complete review of all the reported cases of pineal tumor in the literature in 1938 these authors found that there were 18 cases of teratoma of the pineal body in which the patient was at or below the age of 15 years and in 10 of these precocious physical and sexual development was present. This would indicate that teratoma, which is not known to be associated with precocious puberty when occurring in other parts of the body, is responsible for precocious puberty more frequently than is pinealoma. This observation is of major importance in considering the question of pineal tumors and disturbed endocrine functions since it indicates plainly that pinealoma produces precocious puberty less frequently than another type of tumor primary in the pineal body.

If the supposition is correct that the pineal body is an endocrine gland and its tumor a functioning one, it is reasonable to expect endocrine changes not only in preadolescent life but after puberty as well. No endocrine changes have been observed in the older patients suggesting hormonal disturbances. The only disturbed endocrine function noted in the adult patients was diabetes insipidus, and this disease was noted in the same incidence in the patients below puberty. Moreover, diabetes insipidus is known to be a syndrome associated with hypothalamic lesions and is frequently seen in patients who have had long-standing hydrocephalus with resulting pressure on the hypothalamus.

From the foregoing comment it is plainly seen that pinealoma is not a functioning tumor and is associated with precocious puberty less frequently than are other types of primary tumor of the pineal body. Yet precocious puberty has been reported more frequently with pineal tumors and even with pinealoma than with tumors occurring in other parts of the brain. This fact is strongly attested by the study of Bing, Globus and Simon,<sup>2</sup> who collected 177 cases of pineal tumor from the literature. In their study 21 of the 41 patients at or below the age of 15 years showed the syndrome of precocious sexual development. Primary pineal tumors are not the only intracranial tumors associated with precocious puberty, for tumors and non-neoplastic lesions involving the hypothalamus are occasionally associated with precocious sexual development even though the pineal body is unaffected. Weinberger and Grant<sup>20</sup> reported such a case and were able to collect 16 other cases from the literature. From their study of hypothalamic lesions associated with precocious puberty and a general consideration of the problem, including pineal tumors, they advanced a reasonably sound hypothesis to explain this remarkable association of precocious sexual development with intracranial tumors. According to Weinberger and Grant,

... Lesions of the posterior portion of the hypothalamus interrupt nerve pathways or interfere with mechanisms which normally inhibit and control the production and release of

20. Weinberger, L. M., and Grant, F. C.: *Arch. Int. Med.* 67:762, 1941.

gonadotropic substances from the anterior lobe of the hypophysis. The excessive liberation of these pituitary substances stimulates the ovaries or the interstitial cells of the testes, as the case may be, to overproduction of their specific principles, the estrogenic and androgenic substances. The latter are the substances known to be responsible for the development of the secondary sexual characters.

This explanation is without experimental proof on several points, yet it does offer a satisfactory working hypothesis. Furthermore, it clearly localizes the mechanism for precocious sexual development in the hypothalamus and not in the pineal body. The work of Bing, Globus and Simon<sup>2</sup> offers further support for the conclusion reached by Weinberger and Grant.<sup>20</sup> In 21 cases of precocious sexual development associated with pineal tumor collected from the literature they found that the tumor invariably involved some adjacent structure, such as the corpora quadrigemina, the thalamus or the floor of the third ventricle. No one of these structures was more frequently involved than the others, so that no definite statement concerning the exact location of the mechanism could be made from their study. For a complete review of the reported pathologic data on hypothalamic lesions and precocious puberty and on the role played by the hypophysis, the reader is referred to the paper of Weinberger and Grant.<sup>20</sup>

From a purely pathologic standpoint pinealoma is a unique and interesting type of tumor because it is an example of an autonomous new growth of a whole tissue. That is to say, it contains the same two characteristic cells found in pineal tissue, which in many instances tend to arrange themselves into a characteristic form resembling the mosaic pattern seen in pineal tissue at the time of birth. This unusual characteristic of pinealoma was best shown in case 7 (table 1), in which the new growth was the most striking example of complete reduplication of normal pineal tissue of all the tumors we have studied. Generally, a tumor with cells showing unmistakable autonomy of growth is composed of neoplastic cells of a single type. The individual cells may show extreme pleomorphism with special and varied types of differentiation as in osteogenic sarcoma, in which chondroblasts, osteoblasts and fibroblasts may be intermingled in the same tumor. To illustrate this point further, if carcinoma of the stomach were to reduplicate gastric mucosa to the same degree as pinealoma reduplicates pineal tissue, that tumor would contain glandular structures composed of well differentiated chief and parietal cells that show a tendency to arrange themselves in parallel rows resembling normal gastric mucosa.

Globus and Silbert<sup>4</sup> have referred to this unusual quality of pinealoma as indicating an autochthonous teratoid type of tumor. The term "teratoid" connotes a type of tumor that may reproduce a whole tissue yet cannot correctly be regarded as teratoma because there are not present cells representing all three germ cell layers, hence the term "teratoid." The word "autochthonous" refers to the fact that the tumor is producing exactly the type of tissue from which it originates.

There are only two other types of tumor to our knowledge that show true neoplasia of two different types of cells and represent autonomous new growth of a whole tissue. They are chorioma, with its syncytial and Langhans types of cells, and thymoma, containing thymocytes and collections of epithelial cells resembling Hassall's corpuscles. Neither one of these, however, reproduces the normal tissue as completely as does pinealoma. Chorioma, for example, does not show arrangement of its cells to form functioning blood sinuses as this is seen in normal placenta. Neither does thymoma exactly reproduce normal thymic tissue, for the epithelial cells are usually scattered throughout the tumor in small groups that do not exactly reproduce Hassall's corpuscles. That the ability of

these tumors to produce two types of cells is a fundamental and inherent quality is shown by the fact that their metastases in distant organs show the same characteristic two types of cells.

The remarkably high incidence of pinealoma in males (53 males and only 5 females) is difficult to interpret or explain. There is no known fact to indicate that androgen could contribute to the development of neoplastic disease in the pineal body although the extremely high incidence of pinealoma in males is itself excellent presumptive evidence. Pinealoma will have to be placed with the other types of tumor that show high incidence in males, such as carcinoma of the lung, carcinoma of the lower lip and carcinoma of the esophagus, to await further investigation.

#### SUMMARY

The term "pinealoma" should be reserved exclusively for those primary tumors of the pineal body that reproduce pineal tissue containing two types of cells, which frequently show the characteristic arrangement of a mosaic pattern. Seven such tumors are reported here. In all there were the two characteristic types of cells, and in 6, a mosaic pattern was discernible. A detailed histologic study of these tumors is presented.

In a review of the literature, 51 previously reported tumors diagnosed as pinealoma have been collected: To this group we have added the aforementioned 7. The clinical and pathologic features of this series of tumors are analyzed.

The salient features of pinealoma are that it occurs predominantly in males (88 per cent) between the ages of 15 and 25 years (48 per cent of cases) and that it arises in the pineal body and produces its symptoms by obstructing the aqueduct of Sylvius, thus causing internal hydrocephalus. The initial clinical symptoms produced from the blocking of the aqueduct of Sylvius by the tumor are those of increased intracranial pressure—headaches, vomiting and disturbances of vision. Disturbances of vision and loss of the ability to look up are prominent features of the clinical symptom complex and are due to the frequent involvement of the corpora quadrigemina by the tumor. But there is no group of symptoms that may be considered characteristic of pinealoma. The diagnosis must be made with air studies. Precocious puberty was observed in only 3 of the 17 patients at or below the age of 15 years. Diabetes insipidus was a more frequent complication, with 15 of the 58 patients showing this symptom. This study indicates that pinealoma is not a functioning tumor and plays no direct part in the production of precocious puberty. It is concluded that the mechanism for the production of precocious sexual development associated with intracranial tumors is not directly concerned with pineal tumors or with the pineal body itself but is inherent in the midbrain structures (probably the hypothalamus), because all types of tumors primary in the pineal body as well as of tumors in adjacent structures have been at times accompanied by precocious sexual development.

Pinealoma is a unique type of tumor since it is an example of an autonomous new growth of a whole tissue. It may remain localized in the region of the pineal body; it may be disseminated throughout the third and the lateral ventricles, or it may metastasize to the cerebral and spinal subarachnoidal spaces. Because of its location in the roof of the third ventricle and because of its tendency to spread and metastasize through the central nervous system, it offers little opportunity for complete operative removal. The prognosis is poor, and the cases carry a high mortality rate. Death was known to be the final outcome in 55 of the 58 cases collected.

This study offers no support for the concept that the pineal body is some form of endocrine gland.

# ACUTE ULCERATION OF THE ESOPHAGUS WITH ASSOCIATED INTRANUCLEAR INCLUSION BODIES

REPORT OF FOUR CASES

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AND

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Acute ulceration of the esophagus is not uncommonly encountered at autopsy. The ulcers are usually superficial erosions and only occasionally appear to have been present for any length of time. Often they are at the level of the larynx and surrounding cartilaginous structures, and in the cases with this location especially there is frequently a history of a stomach tube having been used for feeding or for relief of distention. Bacterial stains reveal a variety of organisms, and yeasts and fungi are sometimes observed.

Recently, however, we have encountered 4 cases of acute esophageal ulceration which differ from the common variety in that well formed intranuclear inclusion bodies similar to those associated with the lesions of virus infections could be found in the epithelium bordering on the ulcer. Gram, methylene blue and Giemsa stains failed to reveal bacteria or other visible microscopic organisms. Since the presence of inclusion bodies and the accompanying characteristic nuclear alteration are such good presumptive evidence of the viral origin of these ulcers, it seems worth while to report this unusual occurrence.

## REPORT OF CASES

CASE 1.—A 30 year old white man was admitted to the Long Island College Hospital March 22, 1942. Five months before, he had begun to have abdominal cramps and diarrhea. The stools increased in frequency until he was having from ten to fifteen a day. They were watery and contained blood and mucus. On his admission blood-stained fecal fluid was oozing almost constantly from the rectum. Four weeks earlier he had passed a large amount of fresh red blood and had spent two weeks in another hospital, where he received twelve transfusions. The stools contained no evidence of *Endamoeba histolytica*. The colon bacillus and an unidentified member of the *Salmonella* group were cultured repeatedly in large numbers. Cultures were negative for typhoid, paratyphoid A or paratyphoid B bacilli and the dysentery group. Widal tests were negative.

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The patient was in an extreme degree of emaciation and cachexia when he entered the hospital, and he died nine days later. The diagnosis was nonspecific chronic ulcerative colitis.

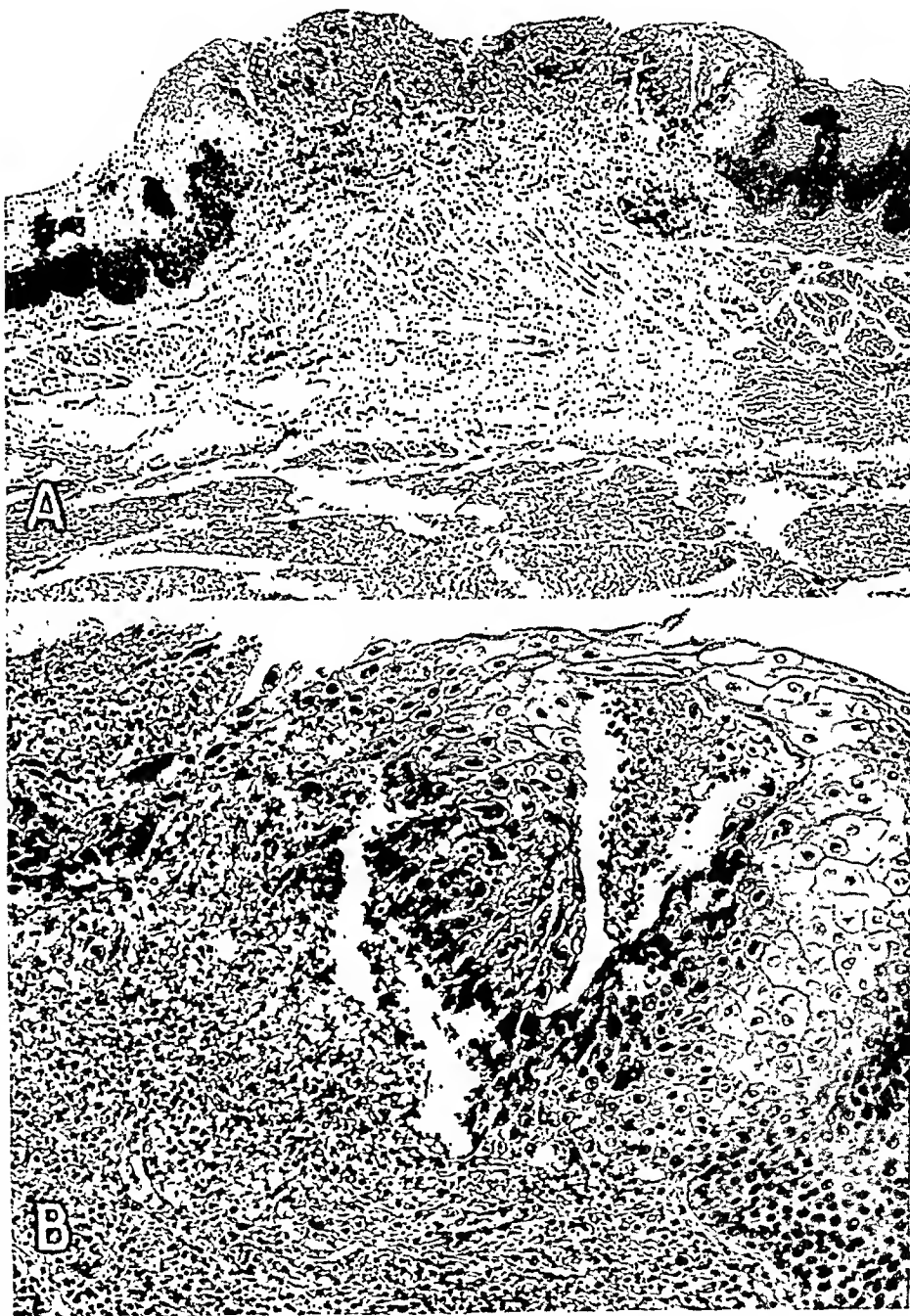


Fig. 1.—*A*, esophageal ulcer in case 1. Hematoxylin and eosin;  $\times 35$ . *B*, margin of the ulcer in *A*. Intranuclear inclusion bodies can be made out in the zone of pale swollen epithelial cells at the border of the necrotic region, especially in the deeper part of the epithelial layer. Hematoxylin and eosin;  $\times 150$ .

Autopsy was performed four and one-half hours after death. Extensive ulceration was present throughout the entire colon, beginning at the ileocecal valve and extending to the rectum. It was more pronounced in the cecum, the transverse colon and the descending colon, where only a few shreds of mucous membrane remained. In many areas the ulceration extended through the muscular coat of the intestine. Lying over the bladder and anterior to the rectum was a well localized abscess containing 100 cc. of thick yellowish green purulent material. Culture of this pus and of the colonic ulcers again revealed only the colon bacillus and the same unidentified member of the *Salmonella* group which had been found in the stools. It was not possible to demonstrate a point of perforation in the intestinal wall which might have given rise to the pelvic abscess.

A moderately extensive lobular or bronchial pneumonia was found in both lungs.

The esophagus was lined by longitudinally wrinkled, pale pink mucous membrane. In the lower 4 cm. of the mucosal surface, especially along the apexes of the wrinkles, there were superficial rounded ulcers, 1 mm. to 4 mm. in diameter, yellow and apparently covered with fibrinous exudate.

Microscopically, the mucosal erosions of the esophagus were found to extend only through the epithelium (fig. 1A). The intervening space was filled with a network of fibrin, in the meshes of which lay necrotic cell fragments, large mononuclear cells, lymphocytes and rare polymorphonuclear leukocytes. At the base of the ulcer there was a small amount of granulation tissue with a peripherally decreasing lymphocytic infiltration. The squamous epithelial cells at the margins of the ulcer stained more palely than those in the unaffected mucosa (fig. 1A and B), and in the nuclei of many of them there were inclusion bodies (fig. 2). These bodies took a dark purplish red stain with hematoxylin and eosin. They were round or oval, conforming in shape to that of the nucleus in which they were contained. At the periphery of the centrally located body there was a clear zone which took no stain. The nuclear membrane was dark, and distinct, and the remaining chromatin was concentrated immediately inside of it, sometimes giving its inner circumference a beaded appearance. This arrangement formed the "halo" which characteristically surrounds viral intranuclear inclusions. In other cells the inclusion filled the entire nucleus, making it a homogeneous purplish red body, around which was the dark and frequently beaded nuclear membrane.

No bacteria could be found in the esophageal ulcers with Gram, Giemsa or methylene blue stains except in the most superficial part of the exudate, where there were rare gram-positive cocci. Cocci and bacilli were abundant in the colonic ulcerations, but a protracted search failed to reveal inclusion bodies in the lesions in the colon or in the remaining mucous membrane. There were neither ulcers nor inclusions in the stomach. The routine histologic sections of heart, aorta, lung, spleen, liver, pancreas, adrenal, kidney, prostate and testis contained no inclusions. The salivary glands were not examined. The final pathologic diagnosis was chronic ulcerative colitis, pelvic peritoneal abscess, bronchopneumonia and acute ulcers of the esophagus.

CASE 2.—A 36 year old white man was admitted to the Long Island College Hospital March 17, 1942. He had been hospitalized four years before because of a spontaneous subarachnoid hemorrhage, from which he recovered without sequelae. Otherwise he had always been in good health. Three days before his final admission he had a sudden attack of severe abdominal pain which, although generalized, was somewhat more severe in the left lower quadrant. There was no associated nausea or vomiting. The pain abated somewhat from its original

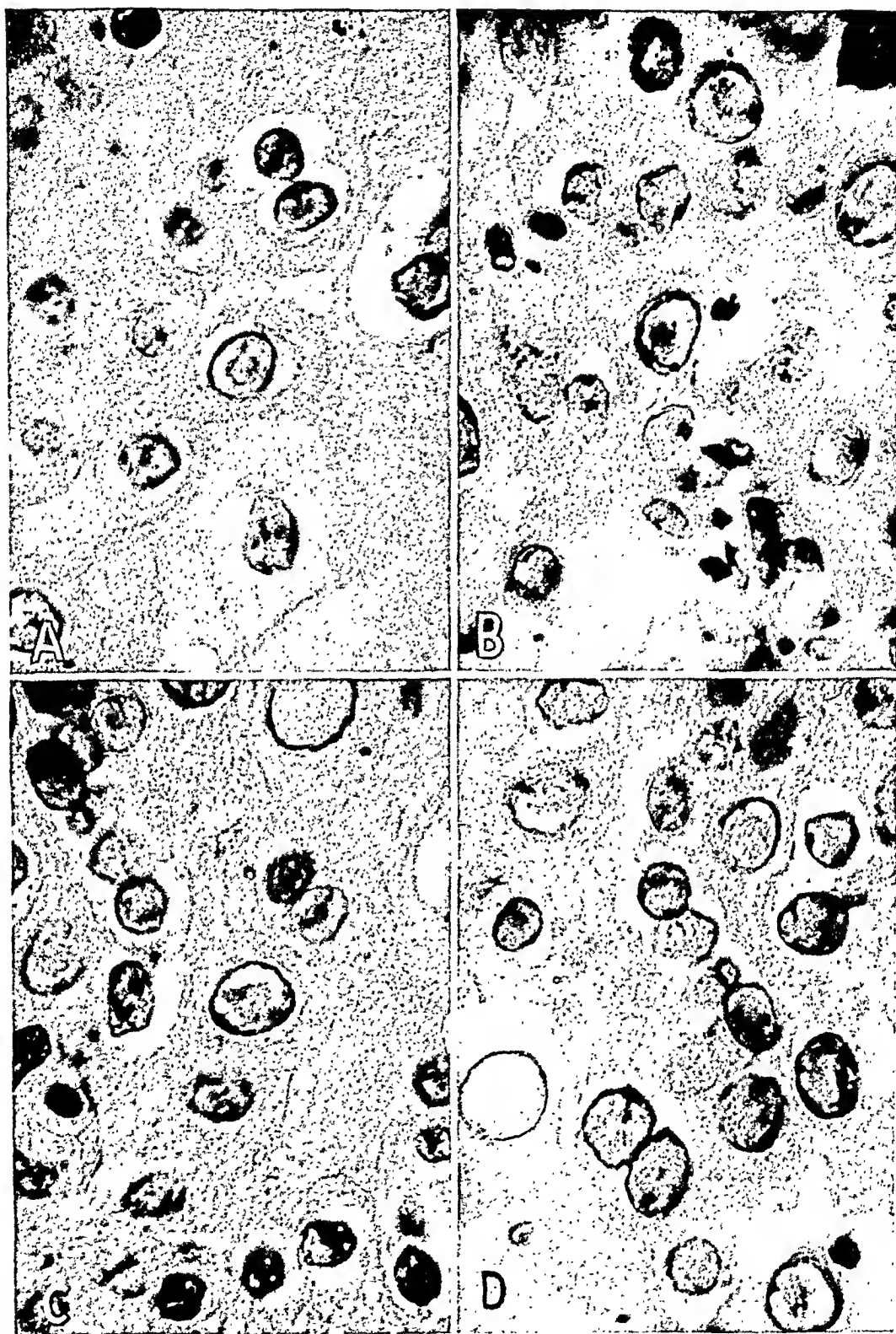


Fig. 2.—Intranuclear inclusion bodies in epithelial cells at the margins of ulcers. Hematoxylin and eosin;  $\times 1,000$ . In *A* and *B* the central inclusion bodies with the surrounding pale zones and beaded nuclear membranes are readily seen. In *C* and *D* there are also many cells in which the inclusion appears to fill the entire space within the nucleus giving it a homogeneous appearance.



severity, but on the third day it again became extreme. He had chills and fever and was brought to the hospital. His temperature was 102.4 F. The pulse rate was 120. Abdominal tenderness and spasm were everywhere marked but were most pronounced in the left lower quadrant. There were 21,000 white blood cells per cubic millimeter of blood, and 90 per cent were polymorphonuclear leukocytes. The diagnosis of generalized peritonitis was made. Operation was thought to be contraindicated. He was treated conservatively and given sodium sulfathiazole (the sodium salt of 2-[paraaminobenzenesulfonamido]-thiazole) intravenously. He remained critically ill throughout the sixteen days of his life in the hospital. During the two days before death he passed several large bloody stools.

Autopsy was performed two hours post mortem. The intestines were bound together by fibrinous adhesions which when separated exposed several large and small pockets of gray purulent fluid. Approximately 800 cc. of purulent material lay beneath the dome of the diaphragm on the left and surrounded the spleen. Both the pus in the pockets between the intestinal coils and that in the sub-diaphragmatic abscess contained the colon bacillus in pure culture.

The origin of the peritoneal infection was found to be an acutely inflamed and perforated diverticulum in the upper portion of the sigmoid colon. The perforation measured 8 mm. in diameter and communicated directly with the lumen of the intestine. In the necrotic margin of the eroded area lay two small branches of the left colic artery. Although at the time of autopsy these vessels contained thrombi, they were presumably the source of the bleeding into the gastrointestinal tract. An ulcer 1.5 cm. in diameter, with indurated and fibrous base and margins was situated on the lesser curvature of the stomach 4 cm. above the pylorus. There was no blood in the stomach, and no vessels could be found in the base of this peptic ulcer.

Four centimeters below the level of the cricoid cartilage, in the mucous membrane covering the posterior wall of the esophagus there was a superficial ulceration measuring 5 mm. in diameter. The erosion was covered by an abundant and elevated deposit of fibrin, which was easily scraped off.

Microscopically, this ulcer closely resembled those in case 1. Again it extended down to but not into the muscle, and again the degenerating epithelial cells at its margin contained acidophilic intranuclear inclusions lying in a clear space, around which the chromatin was concentrated at the nuclear membrane. Gram and Giemsa stains failed to reveal bacteria, yeasts or fungi. No inclusions were found elsewhere in the gastrointestinal tract or in any of the other viscera.

In this case the main part of the pathologic diagnosis was acute diverticulitis with perforation, generalized peritonitis, chronic peptic ulcer of stomach and acute ulcers of the esophagus.

CASE 3.—A 49 year old white man came to the hospital June 18, 1942 because of fever and severe pain in the epigastrium and the right upper quadrant of the abdomen. On laparotomy he was found to have an acutely inflamed and distended gallbladder. The gallbladder was removed surgically, but the patient's temperature remained elevated, and shortly signs of acute generalized peritonitis developed. Death occurred on the tenth postoperative day.

The postmortem examination was made three hours later. In the abdominal cavity the omentum and the loops of the small intestine were bound tightly together and to the anterior abdominal wall by fibrous adhesions. When these adhesions were separated, numerous abscess cavities containing thick green mucoid fluid were found. Only the colon bacillus was cultured from this pus. The mesentery was twisted on itself and fixed by adhesions. The mesenteric veins



were thrombosed. The lower part of the jejunum and the entire ileum had a dark red to blue-black color. The intestinal wall here was edematous and easily torn. The serosa was covered by fibrin.

Scattered over the mucosal surface of the entire length of the esophagus were small superficial ulcerations, which measured 1 mm. to 3 mm. in diameter. These ulcers were rounded, and on their surfaces lay firmly adherent yellow fibrin.

Histologically, the esophageal lesions were similar to those in the preceding cases. The inflammatory reaction at the bases of the eroded areas, however, was more intense, and leukocytes were spread widely beneath the intact epithelium far from the periphery of the ulcer. In addition there were areas in the esophagus where the epithelial lining was thin and the cells were elongated and basophilic, giving the appearance of regeneration and the healing of earlier ulceration. The epithelial cells at the margins of the denuded areas contained intranuclear inclusion bodies which could not be distinguished from those in cases 1 and 2.

There were no other noteworthy anatomic alterations. The chief part of the pathologic diagnosis was acute cholecystitis, acute generalized peritonitis, volvulus, infarction of the small intestine and acute ulcers of the esophagus.

CASE 4.—A 34 year old white woman was brought to the hospital Oct. 3, 1942 in the final stages of cachexia and starvation. Six months previously she had suddenly begun to have diarrhea, with passage of ten to fifteen watery stools daily, which frequently contained blood and mucus. In the early period of her illness she spent five weeks in another hospital, but her improvement there was so slight that she returned home, where she remained until her terminal admission. In the last two months of her illness the diarrhea diminished considerably, but nausea and anorexia persisted to such an extent that she was eating practically nothing. Extreme anasarca developed, which was thought to be the result of starvation and hemorrhage. The serum albumin was 1.3 Gm. and the serum globulin was 1.9 Gm. per hundred cubic centimeters. There were no amebas in the stools. The patient was obviously moribund on admission. Transfusion was of no avail, and she died nine days later without having been submitted to proctoscopy or other investigative procedure.

The postmortem examination was made four and one-half hours after death. The dependent subcutaneous tissues were so edematous that in some areas, such as the dorsa of the feet, actual fluctuation could be elicited. The peritoneal cavity contained 6,000 cc. of clear watery fluid, and in each pleural cavity there was 5,000 cc. of similar fluid. The colon was extensively ulcerated. In the cecum, the ascending and the transverse colon only shreds and polypoid masses of edematous mucosa remained. In the sigmoid the ulcers were numerous but discrete. The rectum was deep red, but its mucous membrane was intact.

The heart was small. The lungs were atelectatic, and in them there were scattered small areas of pneumonic consolidation. The liver contained much fat.

The lesion in the esophagus was almost identical with that seen in case 1. In the lower 5 cm. of the mucous membrane small erosions varying from barely visible pinpoint lesions to ulcers 3 mm. in diameter were scattered on the surrounding intact squamous epithelial surface. Slightly raised masses of dull yellow fibrin, which could be scraped away easily, covered the small denuded areas.

On microscopic examination the epithelial cells bordering on the ulcers contained intranuclear inclusion bodies identical with those seen in the preceding cases. Bacterial stains failed to reveal organisms. Again, as in the earlier cases, no inclusion bodies could be found in the epithelial cells of the remainder of the gastrointestinal tract or in any other organs.

The pathologic diagnosis was chronic ulcerative colitis, anasarca, fatty liver, bronchopneumonia and acute ulcers of the esophagus.

#### COMMENT

The histologic appearance of the intranuclear inclusion bodies around the lesion in these 4 cases is strong presumptive evidence of the viral origin of the esophageal ulcers. The inclusions and the cells containing them were so similar to those found in known virus infections that morphologically it was impossible to distinguish them. This is the type of inclusion which Cowdry<sup>1</sup> put in his class A, the group which is most certainly associated with viral infection. Of course, the observation of inclusion bodies does not in itself prove that a virus is the cause of the lesion in which they are seen, but with the exception of the experimental work of Olitsky and Harford,<sup>2</sup> in which similar appearing bodies followed subcutaneous injection of certain aluminum and ferric compounds into guinea pigs, there is no other demonstrated cause for their presence.

Both the gross and the histologic appearances of the esophageal lesions in the 4 cases were almost identical. In each the ulceration was superficial and extended for only a short distance beneath the previous limits of the epithelium. The infiltrating leukocytes in each case consisted predominantly of lymphocytes and large mononuclear leukocytes, while polymorphonuclear leukocytes were few and scattered. The type of intranuclear inclusion body in the epithelium bordering on the ulcer was identical in all 4 cases.

The distribution and extent of the lesion varied somewhat however. In cases 1 and 4 ulcers were numerous but were confined to the lower part of the esophagus. In case 2 the lesion was single and was in approximately the midportion of the viscus, while in case 3 many ulcers were scattered over the entire extent of the mucous membrane.

There is a striking similarity in all respects between cases 1 and 4. The duration and the course of the illness were the same in both. In both the condition clinically was typical nonspecific chronic ulcerative colitis, and the colonic and esophageal lesions in the 2 cases were almost identical as to character and disposition when seen at autopsy. The presence of the inclusion bodies in the esophageal epithelium of both of the patients of course suggests the possibility that the virus which presumably caused the erosions in the esophagus may have been responsible for the ulcers in the colon as well. Against this hypothesis is the failure to find inclusions in the remaining colonic epithelium, together with their presence in esophageal ulcers of 2 patients who,

1. Cowdry, E. V.: *Arch. Path.* **18**:527, 1934.

2. Olitsky, P. K., and Harford, C. G.: *Am. J. Path.* **13**:729, 1937; *Proc. Soc. Exper. Biol. & Med.* **38**:92, 1938.

although they had intestinal lesions, did not present the picture of chronic ulcerative colitis. The latter of these two arguments against the etiologic significance of the inclusion bodies is the stronger since in several viral diseases although the lesions are widespread, only one type of tissue may contain inclusions. A notable example of this is vaccinia, in which Guarnieri bodies may appear in the stratified squamous epithelium of the cornea or the skin but not in the visceral lesions.

Patients 1 and 2 were admitted to the hospital within five days of each other and died on the same day. If the inclusions have no etiologic significance in regard to chronic ulcerative colitis, then the possibility arises from this chronologic coincidence that the 2 patients were infected from a common source or one from the other. However, the beds of these patients were in widely separated parts of the hospital, and each was cared for by a different group of orderlies and nurses. In each case the nature of the illness necessitated an extremely restricted diet, but the food for each patient was prepared in the same kitchen.

The infectious nature of the esophageal lesion is also suggested by the fact that a review of the autopsy material from the Long Island College Hospital failed to reveal any inclusion bodies in similarly situated ulcers observed in the past. It seems unlikely that 2 cases would appear simultaneously where none had occurred previously if the cause was not an infectious organism. Against the theory of contagion is the fact that the third patient did not enter the hospital until two and one-half months after the deaths of the first 2, and the fourth was admitted only after a further interval of two months.

It is obvious from the inconsequential nature of the esophageal ulcers that they played little part in the disease pictures presented by the patients. If the viral cause of these lesions is admitted, it seems most probable that the infection occurred incidentally and shortly before death. It is conceivable that each patient may have harbored the virus as a harmless saprophyte and that it became pathogenic only when the "resistance" of the host was lowered by the debility accompanying the end stages of a fatal disease. A similar explanation has been postulated for the spontaneous appearance of labial herpes during febrile illnesses.

Material from 3,300 autopsies at the Long Island College Hospital and from 1,500 autopsies at the Long Island Division of the Kings County Hospital included 38 instances of esophageal ulceration. In none of these could inclusion bodies be found in the lesions. The incidence of ulceration was in all likelihood much higher than these figures would indicate. Until interest was focused on the esophagus, small ulcers there were probably overlooked. It may be, too, that the inclusion bodies are present only for a short period in the initial stage of the disease and later disappear as the lesion increases in size and is complicated by secondary bacterial infection. An analogous disappearance of

inclusions in older lesions occurs in several virus diseases, notably vaccinia, in which the Guarnieri bodies are present only during the third twenty-four hours after inoculation, and in virus III disease, in which the intranuclear inclusions are absent in the advanced stages of inflammation.

A survey of the literature disclosed only 1 other case of esophageal ulceration in which inclusion-bearing cells were associated with the lesion. Hartz and van der Sar<sup>3</sup> reported the observation of a single superficial ulcer in the esophagus of a 48 year old Negro in the Netherland West Indies who died of pulmonary tuberculosis and syphilitic aortitis. Their case differs from those described here in that the nuclei containing the inclusions were those of fibroblasts in the granulation tissue and especially those of the intimal connective tissue cells of venules and capillaries. Inclusions did not occur in the epithelium of the esophagus. The photomicrographs of the inclusions also suggest that Hartz and van der Sar observed a quite dissimilar lesion.

Von Glahn and Pappenheimer<sup>4</sup> and Farber and Wohlbach<sup>5</sup> described cases in which on postmortem examination there were found in salivary glands and viscera cells whose nuclei contained typical viral inclusion bodies. These authors did not describe inclusion-bearing cells in the esophageal epithelium, although a detailed examination of material was made in each instance; nor did they mention inclusion bodies in this situation in their extensive discussion. It has not been possible to find in the literature any description of a lesion in the esophagus similar to that seen in these 4 cases.

#### SUMMARY

Four cases of esophageal ulceration in which intranuclear inclusion bodies resembling those seen in viral infections occurred were encountered in the same hospital during a short period. No similar lesions were found in a review of the earlier autopsy material, representing 4,800 necropsies and 38 cases of esophageal ulcers. The similarity of the inclusions to those known to be initiated by a virus and the sudden and almost simultaneous appearance of 2 of the 4 cases suggest an infectious origin. Although the esophageal ulcers were most probably the result of a terminal and incidental infection in a debilitated patient and have no relation to the primary disease, it is interesting that in 2 of the patients the primary disease was nonspecific chronic ulcerative colitis.

3. Hartz, P. H., and van der Sar, A.: *Genesk. tijdschr. v. Nederl.-Indië* **81**:1310, 1941.

4. Von Glahn, W. C., and Pappenheimer, A. M.: *Am. J. Path.* **1**:445, 1925.

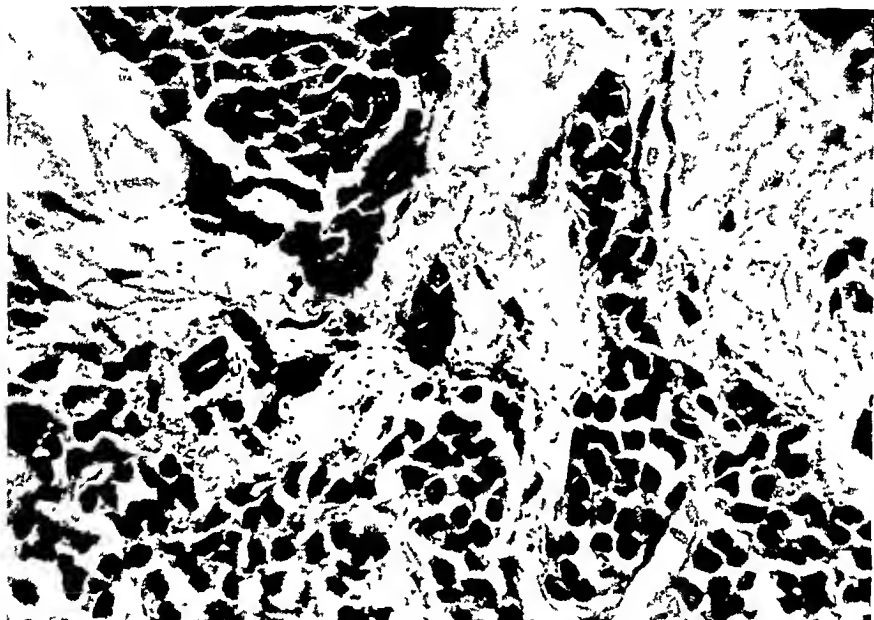
5. Farber, S., and Wohlbach, S. B.: *Am. J. Path.* **8**:123, 1932.

# Case Reports

## CARCINOID TUMOR OF THE GALLBLADDER

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Carcinoid tumors have been found in a number of locations other than the appendix and the small intestine,<sup>1</sup> but a review of the literature reveals only 2 cases of carcinoid tumor of the gallbladder.<sup>2</sup> Both the patients were women, one 64 years old, the other 66 years old, and in each instance the tumor was an incidental finding at autopsy. Following is the report of a third case.



Photomicrograph of gallbladder showing the uniform small cells typical of carcinoid tumors; hematoxylin and eosin;  $\times 660$ .

### REPORT OF A CASE

A 66 year old white woman was admitted to the Western Pennsylvania Hospital, of Pittsburgh, complaining of weakness, general aches, vomiting at intervals and a loss of 40 pounds (18 Kg.) in weight over a nine month period. Roentgenograms following oral administration of dye failed to reveal a gallbladder shadow. Numerous other laboratory procedures gave no significant results.

Ten days after admission cholecystectomy was performed. The thirteenth day after the operation the patient was ambulatory but remained listless and was not

From the Western Pennsylvania Hospital Institute of Pathology (Ralph R. Mellon, M.D., director).

1. Ashworth, C. T., and Wallace, S. A.: *Arch. Path.* **32**:272, 1941.

2. Joel, W.: *Centralbl. f. allg. Path. u. path. Anat.* **46**:1, 1929. Porter, J. E., and Whelan, C. S.: *Am. J. Cancer* **36**:343, 1939.

discharged from the hospital until forty-three days after admission. About ten weeks later she died at home, allegedly of causes unrelated to the gallbladder.

The gallbladder removed at operation measured 15 cm. in length and 3.5 cm. in diameter. The serosa was smooth and glistening. The lumen was filled with many small stones 1 to 3 mm. in diameter and one larger stone, measuring 3.5 by 2.5 by 2.5 cm. The bile was greenish black; the mucosa was trabeculated and ulcerated, and the wall averaged 1 to 2 mm. in thickness. A circumscribed firm yellowish white nodule, 3 mm. in diameter, involved most of the thickness of the wall in the midfundus region, but was covered by both serosa and mucosa.

Examination of sections through the nodule showed small and large masses of fairly uniform, rather small cells with somewhat vacuolated, bluish pink-staining cytoplasm and poorly defined cell boundaries. The nuclei were darkly stained, and although somewhat variable in shape, were usually round or oval. The cell masses were surrounded by a moderate amount of collagenous stroma (figure). Some of the cell masses were separated by clear spaces from the surrounding stroma, and there was some tendency toward formation of clear spaces in the centers of cell masses. The nodule as a whole was not distinctly encapsulated, and cell masses were present just beneath the serosa. The cells showed occasional small argentaffin granules with silver staining and stained red with Masson's trichrome stain. The gallbladder otherwise showed moderate chronic inflammation.

The diagnosis was: carcinoid tumor of the gallbladder and chronic cholecystitis with cholelithiasis.

#### SUMMARY

A case of carcinoid tumor of the gallbladder in a 66 year old white woman is reported. The tumor was an incidental finding in a gallbladder which was filled with stones and showed chronic inflammatory change. This appears to be the third reported case of carcinoid tumor of the gallbladder.

## OSTEOCHONDROBLASTIC MENINGIOMA OF THE LEFT CEREBELLAR HEMISPHERE

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AND

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BROOKLYN

A 62 year old white man, a porter, was admitted to Kings County Hospital to the service of Dr. Jefferson Browder, Jan. 17, 1940. His illness began two and a half months before admission. At that time, while he was working on the docks, some one opened a door behind him, striking the right side of his head. He was dazed and dizzy for a short period afterward. He complained of a dull headache. Two or three days later he noticed that on standing he tended to fall to the right and that on walking he veered to the right. This and the inconstant but daily headache continued. For about six weeks before entering the hospital he complained of progressive weakness of the right leg. January 11, he became restless and confused, taking off and putting on his pajamas, talking with a mumbling speech and appearing quite dull. He was not drowsy but slept more than usual. He had not complained of visual disturbances, but the family noticed that in eating he had difficulty in locating his mouth.

He was well developed and well nourished but appeared ill. The temperature was 99 F.; the pulse rate, 90; the respiratory rate, 28; the blood pressure, 170 systolic and 120 diastolic. He was stuporous, pulled at the covers and removed his shirt. His movements were almost athetoid. He responded to simple commands.

Pupils were constricted, equal, regular and reacted to light. Extraocular movements could not be tested. There was doubtful blurring of the nasal half of the right disk. The teeth were carious; the pharynx, slightly hyperemic; the left tonsil, enlarged and cryptic. Moderate rigidity of the neck was present. The lungs were resonant; a few evanescent rales were heard at both bases. There was no cardiac enlargement; a systolic murmur was heard at the apex. All deep reflexes were present, although sluggish. The right ankle jerk was not obtained. Babinski's signs were not demonstrable. Lumbar puncture gave an initial pressure of 12 mm. of mercury. The cerebrospinal fluid was clear and colorless and contained 6 white cells per cubic millimeter. A Wassermann test of the spinal fluid and one of the blood were negative. Chemical examination of the blood revealed urea 40 mg., creatinine 1.24 mg. and sugar 200 mg. per hundred cubic centimeters. The urine was normal.

January 17, the left upper extremity seemed weaker than the right. The reflexes were more active on the right; the plantar reflexes were flexor in type. January 24, the patient became markedly ataxic and displayed overgroping in reaching for moving objects, with marked loss of check phenomena in the left upper extremity. When the arms were held in extension, the left drifted upward and outward; the right became flexed and dropped to his side. January 28, septic

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fever set in and rose in a few days to 104 F. The patient became unconscious, unable to respond to questioning or painful stimuli or pressure. Pneumonic consolidation of the lower lobe of the right lung occurred, and the patient died suddenly February 5.

January 24, encephalography showed the third ventricle in the midline with no shifting. No evidence of a fracture of the skull was seen. Roentgenograms

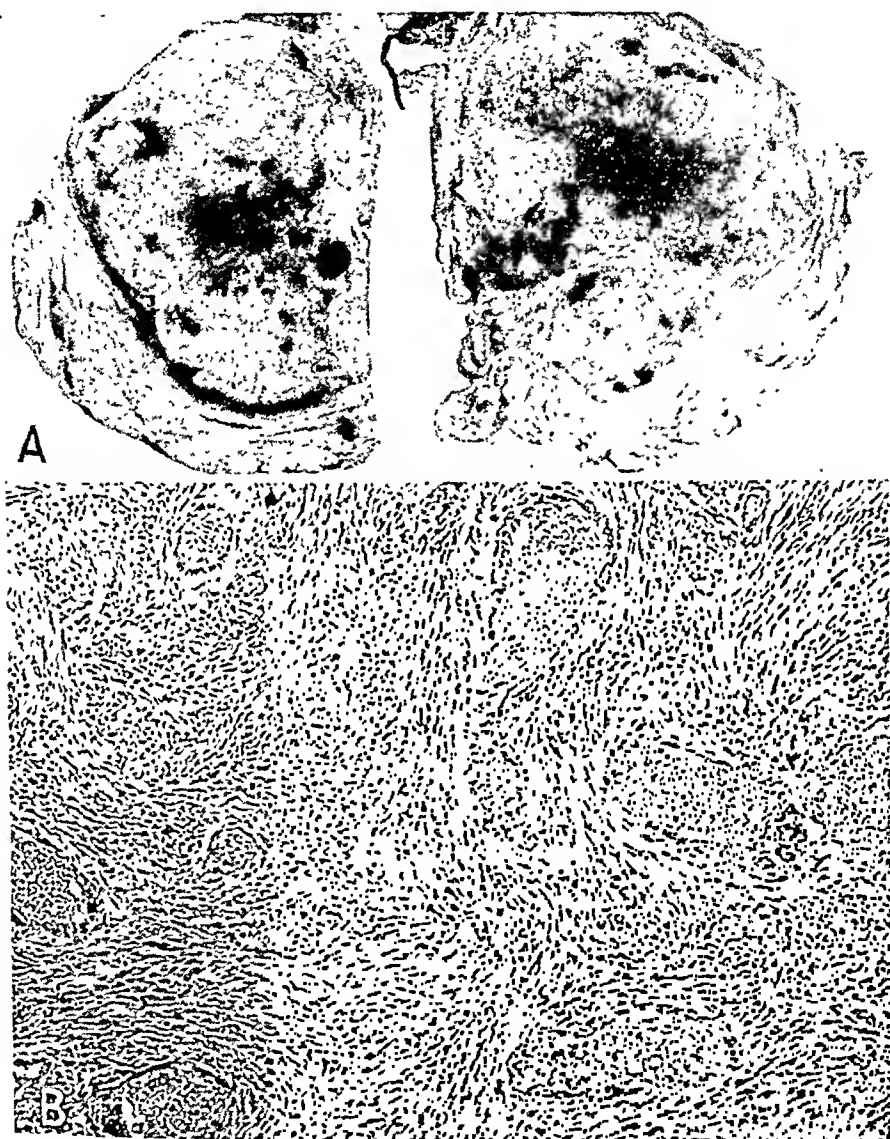


Fig. 1.—*A*, transverse section showing the size and the position of the tumor in the left cerebellar hemisphere. *B*, low power view of the fibroblastic and angioblastic structure of the tumor.

of the skull, January 29, showed air about the convolutions of the brain and a very small amount of air in the third ventricle and the basal cistern. Practically no air had entered the lateral ventricles. A large amount of air was seen in the subarachnoid space and in both parietotemporal regions, indicating some degree of atrophy of the brain. Further studies, January 30, demonstrated an outlined fourth ventricle but no filling of the third or of the lateral ventricles.



The autopsy revealed diffuse bronchopneumonia, chronic passive congestion of the liver, a duodenal ulcer, generalized arteriosclerosis, multiple retention cysts of the kidneys and a small solitary cyst in the tail of the pancreas. No evidence of fracture of the skull or of hematoma was found. The dura was normal except over the left lateral lobe of the cerebellum, where it was adherent to a tumor occupying two thirds of the left cerebellar hemisphere. The tumor was pearl-like and glistening, firm, and densely adherent to the underlying cerebellum within which it lay. Over it the dura mater was firmly attached and could not be separated. Between the cerebellum and the left occipital lobe there was a loose free mass, the size and appearance of a pearl, which was firm but not hard.

After fixation in 5 per cent solution of formaldehyde, the brain was studied more closely. Both frontal lobes showed marked atrophy. The vessels of the circle of Willis showed narrowing of their lumens due to calcification. The left cerebellar hemisphere was larger than the right. On the anterior surface a large pearly growth protruded above the surface of the left hemisphere. The cut surface presented a well encapsulated round mass, 4.5 by 4.5 cm. (fig. 1 *A*), grayish brown and firm. There were scattered pea-sized black areas. The cerebellar tissue formed a 1 cm. ring about the tumor. Section through the tumor revealed small pea-sized areas which were bony-hard and calcific. The tumor did not press on the pons or the medulla. The ventricular system was uniformly dilated. Pinhead-sized cysts were found in the choroid plexus. From the standpoint of histology, the essential features of the tumor were areas of fibroblastic and mesothelial cellular tissue (fig. 1 *B*) with interspersed cartilage and adult bone. There were scattered circumscribed areas of cords of flat cells, some of which had a whorl-like arrangement with small blood channels in their centers (fig. 2 *A*), not unlike the leptomeningioma of arachnoid type described by Globus.<sup>1</sup> Endothelial cells and blood vessels of variable number and in different stages of differentiation were noted. The most striking tissue was new bone in all stages of formation, both membranous, in a matrix of fibroblastic tissue, and cartilaginous.

Some areas showed a transition from fibroblastic tissue to cartilage and then to bone. Many multinuclear giantlike cells were present near capillaries surrounding foci of bone. An occasional giant cell nested in a concavity along the edge of the osseous tissue (fig. 3 *B* and *C*). No psammomma bodies were found. There was a small amount of calcareous deposit about some blood vessels.

In view of the short history one could exclude the possibility of a cerebellar hemorrhage with secondary calcification and bone formation. Moreover, there was no evidence of hemorrhage, of pigment or of scar formation. As to teratoma, Ewing<sup>2</sup> found only two reports, one by Saxer and the other by Eberth. Saxer found a complex teratoid tumor containing cartilage, bone, muscle, chordal tissue, glandular alveoli, cysts lined by cylindric, ciliated or squamous epithelium, pigmented retinal epithelium, choroid plexus, fetal brain tissue and ganglions. Eberth described a tumor, connected by a pedicle with the dura, which was composed of fat, muscle, lymphoid tissue and nerves. In our tumor only osteoid tissue, cartilage and proliferating vascular elements were found.

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1. Globus, J. H.: *A. Research Nerv. & Ment. Dis., Proc.* (1935) **16**:210, 1937.

2. Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1940.

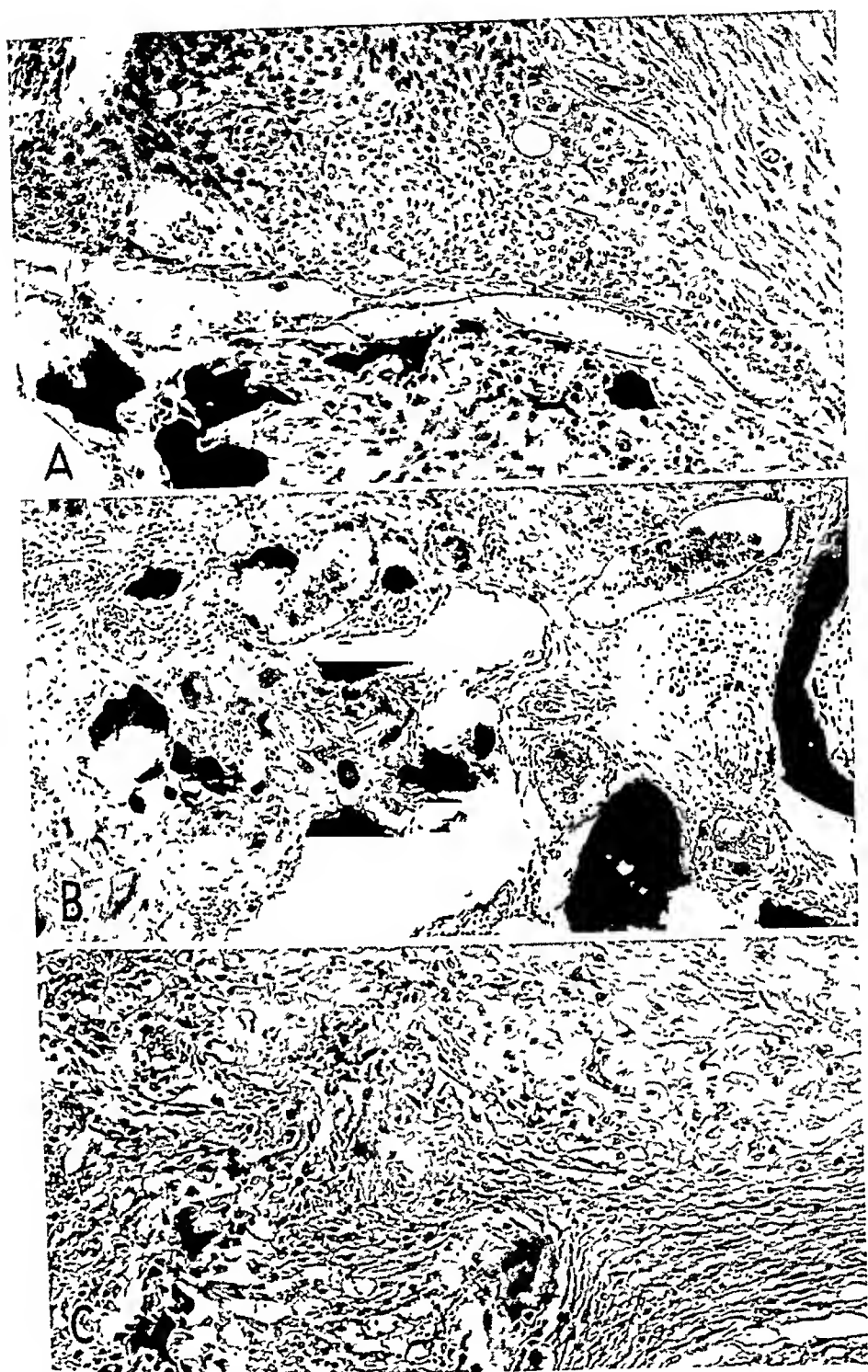


Fig. 2.—*A*, area of whorl-like cell groups. The lower half of the photomicrograph illustrates osteoblastic tissue. Hematoxylin and eosin;  $\times 100$ . *B*, numerous areas of bone and capillary formation with interspersed multinuclear giantlike cells. Calcium is deposited about the blood vessels. Hematoxylin and eosin;  $\times 100$ . *C*, photomicrograph demonstrating the transition from fibroblastic tissue to cartilage and then to bone. Hematoxylin and eosin;  $\times 100$ .

Tumors attached to the dura generally originate from the dura or from the arachnoid. Bailey and Bucy,<sup>3a</sup> Globus<sup>1</sup> and others have demonstrated the multipotentiality of tumors of meningeal origin, which was also fully recognized by Cushing and Eisenhardt.<sup>4</sup> Alpers<sup>5</sup> reported a tumor diagnosed as osteochondroma which he believed arose from a fibroblastic tumor of meningeal origin. He described two additional tumors attached to the choroid plexus. Wolf and Echlin<sup>6</sup> reported a tumor diagnosed as osteochondroma which was attached to the falx and which in their opinion was not meningeal in origin.

Our tumor was not unlike the reported ones, but it contained an area characteristic of meningioma, which establishes beyond doubt its meningeal origin.

The source of the bone and the cartilage in these tumors has been the topic of much dispute. That bone may develop in the meninges is borne out by the development of bony plaques in the dura and the falx, with all the characteristics of true bone. This is established further embryologically by Globus, who demonstrated the development of meninges next to primitive mesenchyme prior to the separation of bone and periosteum from the meninges. That cartilage as well may form under these circumstances is the opinion of Alpers<sup>5</sup> (and perhaps others). Although Bailey<sup>3b</sup> denied this possibility, it is based on fact that membranous bones of the skull do not lay down cartilage. If it is accepted that the osteoblastic tumors attached to the dura are meningeal in origin, it must be accepted that cartilage is a part of the same tumor. We believe that in our case the development of bony islands and cartilaginous areas could be traced directly to the fibroblastic tissue.

This tumor, therefore, was one of mixed type exhibiting some of the histologic characteristics of meningioma. It manifested the multipotentiality of the tumors of this class, as demonstrated by its fibroblastic, angioblastic, osteoblastic and chondroblastic features. The last two elements are quite unusual. Apparently, the tumor was of slow growth and not related to trauma. The slow growth probably accounted for the absence of significant symptoms, which is explained by the ability of the brain to accommodate itself to slowly developing pressure.

While tumors of the brain are notorious for the frequency with which they produce atypical signs, those of the posterior fossa usually conform to a somewhat normal pattern. The patient with a tumor of the posterior fossa presents evidence of increased intracranial pressure. Elevation of the spinal fluid pressure and papilledema are almost constant. Grant and associates<sup>7</sup> studied 158 cases of cerebellar tumor which presented unusual symptoms. In this series only 15 cases failed to show papilledema. Lumbar puncture in our case gave normal results. The roentgenogram revealed nothing to suggest a lesion in the posterior fossa.

3. (a) Bailey, P., and Bucy, P. C.: *Am. J. Cancer* **15**:15, 1931. (b) Bailey, O. T.: *Arch. Path.* **30**:42, 1940.

4. Cushing, H., and Eisenhardt, L.: *Meningiomas*, Springfield, Ill., Charles C Thomas, Publisher, 1938.

5. Alpers, B. J.: *Ann. Surg.* **101**:27, 1935.

6. Wolf, A., and Echlin, F.: *Bull. Neurol. Inst. New York* **5**:515, 1936.

7. Grant, F. C.; Webster, J. E., and Weinberger, L. M.: *Am. J. M. Sc.* **202**:313, 1941.

The history of trauma, the fluctuating clinical picture, the absence of any characteristic symptoms with few localizing signs, associated with stupor, pointed to a lesion of traumatic origin. The patient presented difficulties in "locating his mouth," which is generally a symptom of cerebral origin. The results of the study of the encephalograms were likewise misleading and failed to demonstrate the presence of the tumor.

The salient, unusual feature of this case was the presence of a large tumor of the cerebellum with no increase in the intraspinal pressure and with inconstant cerebellar symptoms.

#### SUMMARY

A case is reported of a tumor of the cerebellum, which did not present papilledema or other clinical signs usually associated with a tumor of the posterior fossa. The tumor was diagnosed as meningioma with osteoblastic, chondroblastic and angioblastic features.

## TUMOR OF THE CAROTID BODY AND THE PANCREAS

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In 1891 Marchand first described a tumor of the carotid body. Since that time approximately 275 examples of this type of neoplasms have been reported. The literature has been reviewed by Bevan and McCarthy,<sup>1</sup> Rankin and Wellbrook,<sup>2</sup> Greene and Greene<sup>3</sup> and Peterson and Meeker.<sup>4</sup>

In all the reported instances of tumor of the carotid body the neoplasm has been localized in the neck, never spreading beyond the lymph nodes in the immediate neighborhood of the primary mass. The case reported by Gilford and Davis<sup>5</sup> and that by Mönckeberg<sup>6</sup> are the only ones on record in which autopsy disclosed a tumor outside of the neck. In the former the liver was studded with soft white nodules, and in the latter ovarian tumors were found. In both instances the diagnosis of tumor of the carotid body is doubtful.

In several reported cases both carotid bodies have been involved, either simultaneously or one after the other. One autopsy<sup>7</sup> showed, in addition to the neoplasm of the carotid body, tumors of the organs of Zuckerkandl. These organs are two small bodies of chromaffin tissue which lie on the anterior surface of the abdominal aorta at the origin of the inferior mesenteric artery. They are conspicuous in fetuses from 5 months to full term, after which they rapidly undergo atrophy. From the second year of life on, they are extremely difficult to find.

The present report records observations on a patient with a tumor of the carotid body in whom a nodule histologically identical with the mass in the neck was present in the body of the pancreas.

### REPORT OF A CASE

A 47 year old Negro entered Barnes Hospital in 1927 for treatment of a mass in the left side of the neck. The mass had been present for five years, and in the few months prior to his admission to the hospital had increased in size. The Wassermann reaction of the blood was 4 plus, and a history of a primary syphilitic lesion occurring twenty-five years before was elicited. The mass, which did not pulsate, was considered to be a gumma, and the patient was given anti-syphilitic treatment, which he discontinued after two months. For the following fifteen years he felt well, and the mass in his neck, although increasing constantly in size, caused him no discomfort.

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From the department of pathology and surgery of Washington University School of Medicine and Barnes Hospital.

1. Bevan, A. D., and McCarthy, E. R.: Surg., Gynec. & Obst. **49**:764, 1929.
2. Rankin, F. W., and Wellbrook, W. L. A.: Ann. Surg. **93**:801, 1931.
3. Greene, E. I., and Greene, J. M.: Am. J. Surg. **22**:521, 1933.
4. Peterson, E. W., and Meeker, L. H.: Ann. Surg. **103**:554, 1936.
5. Gilford, H., and Davis, K. L. H.: Practitioner **73**:729, 1904.
6. Mönckeberg, I. G.: Beitr. z. path. Anat. u. z. allg. Path. **38**:1, 1905.
7. Cragg, R. W.: Arch. Path. **18**:635, 1934.

May 7, 1942, he was readmitted to Barnes Hospital. He was now 62 years of age. He gave a history of dyspnea, orthopnea, paroxysmal nocturnal dyspnea, edema of the ankles and enlargement of the abdomen, all of fourteen months' duration. Sharp substernal pain was also present. He had been treated in another hospital twice during the past year for the same complaints, with slight improvement following rest in bed and digitalis therapy. The mass in his neck had now been present for twenty years and caused no discomfort. It was twice as large as it had been fifteen years before, and measured 12 cm. in diameter.

On admission the patient was moderately dyspneic and orthopneic. There was edema of the ankles and over the sacrum. The veins of the neck were distended. The pupils were small and reacted sluggishly to light. There was a firm mass, 12 cm. in diameter, below the angle of the left mandible. It was freely movable laterally but fixed vertically. This mass was thought to have expansile pulsation and was considered to be an aneurysm of the left carotid artery. The heart was enlarged, the apex impulse being seen and felt in the anterior axillary line. There was a soft, short systolic murmur at the apex. The aortic second sound was hollow, high pitched and loud, with a short diastolic murmur heard best in the second right intercostal space. The blood pressure in each arm was 170 systolic and 70 diastolic. The liver was enlarged and slightly tender. The peripheral arteries were moderately thickened. The knee jerks and ankle jerks were absent; otherwise the neurologic examination showed nothing abnormal.

There were 4,470,000 erythrocytes and 7,100 leukocytes per cubic millimeter of blood. The hemoglobin level was 70 per cent. The differential percentages were: stab cells, 6; segmented neutrophils, 66; lymphocytes, 25, and monocytes, 3. The urine contained a trace of albumin. The Wassermann reaction of the blood was 4 plus. The nonprotein nitrogen of the blood amounted to 23 mg. per hundred cubic centimeters. Examination of the spinal fluid by lumbar puncture revealed normal pressure and dynamics. The Pandy test and the Wassermann test of the fluid were positive. The colloidal gold curve showed a midzonal reaction, and the total protein of the fluid was 47 mg. per hundred cubic centimeters. Kymographic roentgen examinations revealed the heart to be moderately enlarged. There was an increase in the excursions, especially over the right side of the heart, and the aorta filled and emptied rapidly. These findings were interpreted as indicative of aortic regurgitation.

Treatment with digitalis and diuretics resulted in no improvement in the condition of the patient. He began to complain of severe headaches, nausea and vomiting. No explanation for these symptoms was found. On the eighteenth day in the hospital, the pulse rate suddenly fell to 40 per minute, and an electrocardiogram revealed auricular fibrillation and complete heart block. This condition persisted until death. Ten days later, the temperature rose to 39.3 C. (102.7 F.), and the patient became semicomatose. The breath had a uremic odor. Fluids were administered parenterally, and feeding was begun by stomach tube. Severe diarrhea developed which persisted until death. He was comatose for the last twenty-eight hours of life. At this time the blood nonprotein nitrogen was 210 mg. per hundred cubic centimeters. The patient died June 7, 1942, thirty-three days after his admission.

*Autopsy* (twelve hours post mortem).—The heart was large, weighing 680 Gm. There was advanced syphilitic involvement of the ascending aorta with widening of the commissures, of the aortic valve. There was moderate chronic passive congestion of the lungs, the liver and the spleen. The lower lobes of the lungs

were partially consolidated, and there was purulent bronchitis of all the lobes. The wall of the ileum was congested, and the lymphoid tissue of the colon was prominent. Other findings were moderate sclerosis of the aorta and coronary arteries, slight sclerosis of the hepatic, splenic and renal arteries, focal scars of the kidneys and calcification of the renal pyramids. The gastrointestinal tract, the gallbladder, the urinary bladder and the prostate gland were normal.

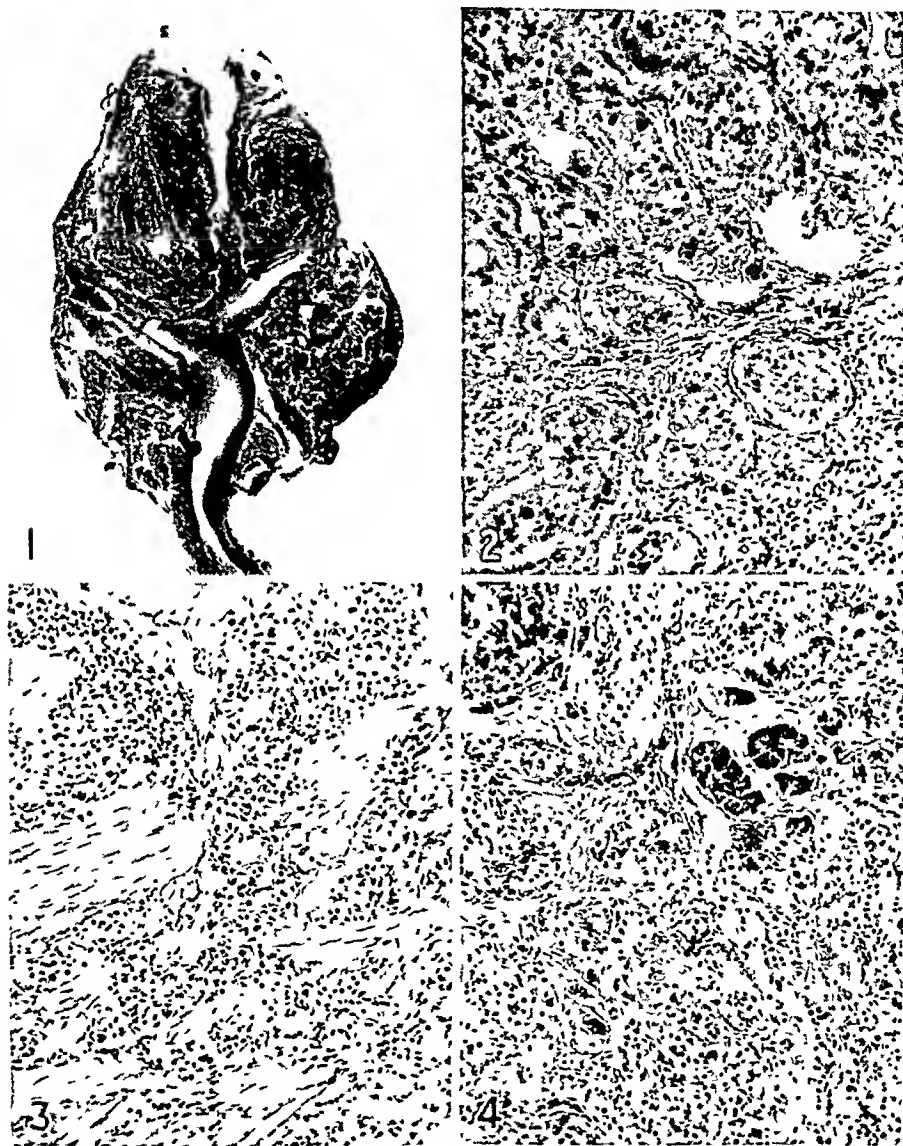


Fig. 1.—Cross section of the tumor of the carotid body. The common, internal and external carotid arteries are surrounded by tumor ( $\frac{3}{8}$  original size).

Fig. 2.—A section of the tumor of the carotid body showing the alveolar arrangement of the tumor cells.  $\times 98$ .

Fig. 3.—Wide bands of partially hyalinized fibrous tissue traverse the tumor of the carotid body.  $\times 98$ .

Fig. 4.—A section of the nodule of tumor in the pancreas. Pancreatic acini are present among the tumor cells.  $\times 98$ .

At the bifurcation of the left common carotid artery there was a firm nodular gray-pink mass, measuring approximately 10 cm. in diameter. This mass completely surrounded the internal and external carotid arteries, which were patent but compressed (fig. 1). The cut surface of the tumor was gray-pink and traversed by fine fibrous interlacing trabeculae. The mass was attached only to the carotid arteries, being easily separated from surrounding structures in the neck. There were no nodules of tumor elsewhere in the neck. The cervical lymph nodes were normal in all respects.

In the anterior part of the body of the pancreas, which was otherwise normal, there was a firm spherical gray-pink nodule, measuring 15 mm. in diameter. This mass was not well demarcated from the pancreatic tissue. It was within the substance of the pancreas and not visible on the surface.

Microscopically, the tumor of the neck was composed of moderately large cells with eosinophilic cytoplasm and fairly large vesicular nuclei, which showed moderate variation in size and shape. Most of the nuclei contained one or more prominent nucleoli, and the chromatin was scattered throughout the nucleus in the form of granules. Mitotic figures and multinucleated cells were present in moderate numbers. The tumor cells were arranged in alveolar pattern, supported by fine strands of collagen (fig. 2). Numerous large sinusoids coursed through the tissue, in many areas in close relation to the tumor cells. At no point were there tumor cells within the sinusoids. A moderate number of macrophages containing irregular granules of golden brown pigment was present. There were numerous accumulations of lymphocytes and plasma cells in the tumor. Large broad bands of fibrous tissue divided the mass into large nodules. Much of the connective tissue showed hyaline change (fig. 3). There were no enlarged lymph nodes in the neck, and no evidence of tumor involvement of lymph nodes was found. The tumor in the pancreas was microscopically identical with that in the neck. At one point there was pancreatic tissue within the tumor nodule (fig. 4).

Tissue from each mass of tumor, fixed in solution of formaldehyde, was stained by Schmorl's method<sup>8</sup> in an attempt to identify chromaffin granules. None were found in either tumor. The only other fixative used was a modification of Helly's fluid in which zinc chloride was used instead of mercuric chloride. This tissue had been in alcohol, which made it unfavorable for demonstration of chromaffinity, and although it was stained by Wiesel's technic,<sup>8</sup> no granules were demonstrable.

#### COMMENT

In view of the advanced syphilitic aortitis, the dilatation of the ring of the aortic valve, the enlargement of the heart and the congestion of the lungs, the liver and the spleen, the immediate cause of death was obviously cardiac failure. The history of cardiac decompensation for the year before death bears out this conclusion. Failure to improve on treatment with digitalis and diuretics is the rule in syphilitic aortic valvular disease with regurgitation, and it was true in this case.

The tumor of the left carotid body and that of the pancreas were incidental findings. That in the neck had been present for twenty years. The duration of the mass in the pancreas is, of course, unknown.

The tumor in the neck was grossly and microscopically characteristic of tumor of the carotid body as that condition has been described in the

8. Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938, pp. 267-268.



literature. The tumor in the pancreas was histologically identical with the mass in the neck. Since such a tumor has not been hitherto described in the pancreas, a consideration of the relation of the two neoplasms must necessarily be inconclusive.

The tumor in the pancreas must be either a metastasis from the mass in the neck or a separate neoplasm illustrating multicentric origin of neoplasm in similar tissues. There is normally no tissue in the pancreas which is similar in structure to the carotid body, from which the tumor in the pancreas might have arisen. However, it is well recognized that there is an abundance of chromaffin tissue along the abdominal aorta. It is possible that the tumor in the pancreas was derived from ectopic chromaffin tissue which had at some time become incorporated in the pancreas.

Tumors which spread widely occasionally metastasize to the pancreas, although this is not especially common. No neoplasm of the carotid body has ever been known to spread beyond the lymph nodes of the neck. In this particular instance, even these lymph nodes were uninvolved. It is therefore extremely unlikely that the nodule in the pancreas is a metastasis from the tumor of the carotid body. The idea of multicentric origin of neoplasm most satisfactorily explains the origin of the tumor of the pancreas.

The failure to demonstrate the presence of chromaffin granules in both tumors was not unusual. The fixation of the tissues was not optimal for the preservation of these granules. In those instances in which studies of chromaffinity have been carried out, there have been as many negative as positive results. It must be concluded that the chromaffin property of this type of tumor is an inconstant one.

Tumor of the carotid body has been studied by others for the presence of epinephrine, and in no instance was there a positive reaction. This is in agreement with the clinical findings, in that no disturbances have been observed in patients with this tumor, other than those directly related to the size of the mass or to the diminution in the supply of blood to the brain on the involved side. No relation exists, therefore, between the presence of chromaffin granules and that of epinephrine in these tissues.

Consideration of the incidence of tumor of the carotid body throws little light on the cause of the lesion. The patients have been between 7 and 73 years of age, the majority being between 40 and 60 years. They are about equally divided between the two sexes. No racial preponderance has been observed. Occurrence of the tumor in Negroes has been described. The average duration of the tumor when first seen by a physician is about seven years. In some cases that have been described the duration was thirty-seven years.<sup>6</sup> A study of the literature reveals no relation to any other recognized disease.

Numerous names have been applied to the tumor arising in the carotid body, among them being "perithelioma," "endothelioma," "paraganglioma," "ganglioneuroma," "angioma," "angiosarcoma," "neuroblastoma," "carcinoid," "sympathetic nevus," "carotid tumor" and "chromaffinoma." A tumor is properly named on the basis of the cell of origin rather than on that of the organ of origin or that of the structure of the tumor. The carotid body is generally believed to arise from the sympathogonia, cells of ectodermal origin, which are considered

to be the precursors of the sympathetic and chromaffin systems. The small masses of chromaffin tissue which are scattered along the abdominal aorta, and also the carotid body, are called paraganglions. The cells of which they are composed are more properly called chromaffin cells, and a tumor of any of these masses should be called a chromaffinoma. It is of interest to note that the "Standard Nomenclature of Disease" recognizes only the names of "benign or malignant tumor of the carotid body."

#### SUMMARY

A tumor of the carotid body associated with a histologically identical tumor in the pancreas is reported. The question of metastasis or multicentric origin is discussed. The various terms by which these tumors are known are mentioned, and "chromaffinoma" is selected as the most appropriate.

## General Reviews

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### PERMEABILITY OF THE BLOOD-BRAIN BARRIER TO NEUROTROPIC VIRUSES

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In a recent review of the literature on the problem of the blood-brain barrier I arrived at the following conclusions: 1. The term "blood-brain barrier" expresses a selective permeability of the capillaries of the central nervous system. 2. The ability of aniline dyes, toxins, antibodies and drugs to pass the walls of these capillaries is determined by the electrical charge on each of these substances. 3. The capillaries of the central nervous system are permeable to electropositive and electroneutral substances but impermeable to electronegative ones.

In view of the almost unlimited number of substances to be considered, the permeability of the blood-brain barrier to neurotropic viruses was discussed only in a condensed form. The practical importance of the subject, as well as the large number of interesting observations pertaining to it, justifies a more extensive review of the literature. Moreover, it will be seen presently that opinions concerning the permeability of the blood-brain barrier to neurotropic viruses are still widely divergent. In an excellent article on neurotropic viruses Hurst expressed himself as follows: "The limited evidence available suggests that viruses do not easily pass the barrier." In a subsequent passage of the same article he was even less reserved when he wrote: "From a pathogenetic point of view the significance of virus in the blood lies not in the immediate consequences, for the normal haematoencephalic barrier is impervious to most if not all viruses." An entirely different view has been taken by Doerr, who emphatically discarded the concept that the capillaries of the central nervous system are impermeable to neurotropic viruses.

The fact that two authors equally conversant with the subject arrive at conclusions so widely divergent raises the question whether the criteria for permeability of the blood-brain barrier to neurotropic viruses have always been visualized with sufficient clarity. It is now the consensus that the majority of neurotropic viruses, if not all of them, are able to reach the central nervous system along neural pathways. This fact alone, however, cannot be considered as evidence that they are unable

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to pass through the capillaries of the central nervous system. In the first place it must be shown that the neural pathways are the only routes by which neurotropic viruses reach the central nervous system. In the second place it must be ascertained whether or not virus injected into some peripheral tissue reaches the capillaries of the central nervous system through the circulation.

In that respect neurotropic viruses fall into two distinct groups. Examples of those belonging to the first group are the viruses of pseudorabies, louping ill, equine encephalomyelitis and St. Louis encephalitis. These viruses are not strictly neurotropic. They invade the blood stream and may circulate for several days. If, nevertheless, they can be shown to reach the central nervous system exclusively along neural pathways, it may safely be assumed that they are unable to pass through the capillaries of the central nervous system.

The problem is more complicated in the case of the strictly neurotropic viruses, such as the viruses of poliomyelitis, rabies and Borna disease. These viruses when injected into an extraneural tissue do not invade the blood stream in any appreciable amounts. Experiments of this type, therefore, give no clue as to whether or not these viruses are able to pass the capillaries of the central nervous system. The only possibility of obtaining an answer to this question is given by the intravenous injection of large amounts of these viruses. If the injected virus fails to infect the experimental animal by the intravenous route, its inability to pass the capillaries of the central nervous system may be considered as proved. If infection takes place, several possibilities must be taken into consideration. The central nervous system may be invaded either directly through the capillary walls or the virus may leave the vascular system and enter nerve endings in some extraneural tissue. In the first case further experiments are necessary to determine whether the permeation is due to physicochemical forces or to multiplication within the capillary endothelium. The decision as to whether or not the capillaries of the central nervous system are permeable to an individual neurotropic virus therefore requires an answer to the following questions:

1. Is the virus able to reach the central nervous system along neural pathways?
2. Does it reach the central nervous system exclusively by this route?
3. Does the virus invade the blood stream?
4. If not, does it reach the central nervous system after intravenous injection?
5. If it reaches the central nervous system by the intravenous route, does it pass the capillaries of the central nervous system or does it first leave the vascular system and reach the central nervous system indirectly along neural pathways?

6. Where direct passage through the capillary walls can be demonstrated, the mechanism of permeation must be further investigated.

In the following sections the literature on neurotropic viruses will be reviewed from these points of view. It will be seen that each virus must be studied on its own merits. There is considerable variation in the mechanisms by which the individual neurotropic viruses reach the central nervous system.<sup>1</sup> Part of the diversity of opinion concerning the permeability of the blood-brain barrier is apparently due to the circumstance that findings holding true for one particular virus have been generalized for other viruses.

The question may be asked whether it is worth while carrying out all these complicated investigations only to determine whether viruses are able to pass the blood-brain barrier. Particularly in the case of the strict neurotropic viruses it may be argued that their transmission by neural pathways is all one needs to know concerning their penetration to the central nervous system. Actually, however, the investigations listed are indispensable if one wants information concerning the factors that determine the ability or the inability of neurotropic viruses to pass the blood-brain barrier. What those factors may be will be discussed in the second part of this review.

#### PERMEABILITY OF THE BLOOD-BRAIN BARRIER TO INDIVIDUAL NEUROTROPIC VIRUSES

*Poliomyelitis.*—In recent years the clear picture of the genesis of poliomyelitis as it revealed itself in experiments on *Macacus rhesus* has undergone some modifications. It has become quite doubtful whether the nasal mucous membrane can be considered as the portal of entry in human beings. An ever increasing number of investigators are inclined to attach more importance to the intestinal route. The cynomolgus monkey and the green African monkey have been shown to be susceptible to this mode of infection. Moreover, it will be seen in later paragraphs that the manner in which virus spreads in the animal body may be different for individual strains of the virus of poliomyelitis. At present it is difficult to judge whether these findings have any bearing on the question of the permeability of the blood-brain barrier to the virus of poliomyelitis. The following discussion will be confined to experiments in the monkey (*M. rhesus*) and to strains passed for a considerable time through this species.

It has been shown beyond doubt that the virus of poliomyelitis reaches the central nervous system by neural pathways. Flexner and Lewis, Landsteiner and Levaditi, Leiner and von Wiesner (1910 c) and many

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1. Moreover, the species and the age of the experimental animal are important factors.

others have found that monkeys can be infected by the intranasal route. Three or four days after inoculation the virus was found exclusively in the olfactory bulbs and tracts (Levaditi and Landsteiner; Flexner and Clark; Sabin and Olitsky, 1938; Faber and Gebhardt). The same observation was made by Sabin and Olitsky (1936-1937) with the aid of histologic methods. Killing the experimental animals on successive days after inoculation of the virus and studying the pathologic changes of the central nervous system, Fairbrother and Hurst, as well as Faber, reached the conclusion that from the olfactory region the virus spreads to the hypothalamus, to the thalamus and, through the spinothalamic tract, to the lumbar cord. The spread of the virus within the central nervous system has recently been studied in greater detail by Howe and Bodian. The interested reader may be referred to their excellent monograph. The spread of the virus in these anatomic structures shows clearly that the virus after intranasal inoculation reaches the central nervous system neither through the circulation nor the cerebrospinal fluid. This conclusion is further substantiated by the fact that monkeys subsequent to removal or destruction of the olfactory bulbs or tracts can no longer be infected by the intranasal route. According to Armstrong and Harrision, Schultz and Gebhardt (1936) and Sabin, Olitsky and Cox, instillation of aluminum sulfate, nitrophenol, trinitrocresol, mercurochrome or zinc sulfate has the same inhibitory effect.

Flexner and Lewis, Leiner and von Wiesner (1910 c) and Hurst produced poliomyelitis by injecting the virus into the sciatic nerve. Since Horster and Whitman showed that fluids injected into the nerve may reach the cerebrospinal fluid through artificial channels, it is questionable whether the experiments of the aforementioned authors are entirely conclusive in demonstrating the migration of the virus in the sciatic nerve. Recently Bodian and Howe reported interesting experiments which meet this objection. They were able to produce poliomyelitis by dipping the central end of the cut sciatic nerve into suspensions of the virus. They further made the important observation that the result was negative after the axis-cylinders had been destroyed by local application of solid carbon dioxide. According to Toomey, the virus of poliomyelitis is transmitted to the central nervous system essentially through the unmyelinated fibers of the sympathetic nervous system.

It is more than questionable, however, whether the virus after peripheral inoculation reaches the blood in any appreciable amounts. It has never been found in the blood of patients with poliomyelitis or in that of monkeys after intranasal instillation. Even after intracerebral injection it could be identified in the blood only occasionally (Gordon and Lennette).

Whether the virus of poliomyelitis reaches the central nervous system from the blood, therefore, can be decided only by experiments

in which it is injected by the intravenous route. Clark, Fraser and Amoss have shown that monkeys cannot be infected by this route unless overwhelming doses are given. This is significant since even seventy-two hours after intravenous injection the virus was still found in the blood.

Clark, Fraser and Amoss obtained positive results with repeated intravenous injections of large doses of the virus (approximately 1,250 intracerebral minimal lethal doses). Even under these conditions, however, the virus did not reach the central nervous system directly by way of the circulation. Lennette and Hudson (1935) and Armstrong showed that monkeys cannot be infected by the intravenous injection of the largest doses after section of the olfactory tracts or chemical treatment of the nasal mucous membrane. These results can be interpreted only on the assumption that the virus after intravenous injection is excreted on the nasal mucous membrane where it is taken up by the olfactory nerve endings. On the other hand, Lennette and Hudson (1936) reported that the intravenous injection of even relatively small and otherwise ineffective doses of virus produced poliomyelitis when the cerebral vessels had been damaged by an intracerebral injection of a sterile solution of starch. Therefore the experimental evidence shows convincingly that the virus of poliomyelitis is unable to pass the normal capillaries of the central nervous system.<sup>2</sup>

*Rabies.*—In its specific affinity for nerve tissue the virus of rabies closely resembles the virus of poliomyelitis. As early as 1887 di Vestea and Zagari showed in a series of classic experiments that the virus of rabies reaches the central nervous system by way of the peripheral nerves. They injected the virus into the sciatic and median nerves, respectively, and observed that paralysis started in the legs innervated by the injected nerves. Moreover, after intrasciatic inoculation the virus first appeared in the lumbar cord, whereas after injection into the median nerve the first appearance was in the cervical cord. Transection of the dorsal cord confined the virus to the part first reached from the injected nerve. Helman injected the virus into the tip of the tail. If the tail was removed within twenty-four hours after the injection, the animal remained in good health. Schweinburg and Windholz in experiments on parabiotic rats found that rabies developed only in the rats into which the virus had been directly injected. All these

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2. More recent investigations seem to indicate that the genesis of the experimental disease may be different for various strains of the virus of poliomyelitis. German and Trask found a dermatropic strain highly infective by the intravenous route, even after section of the olfactory nerves. Jungeblut, Finer and Sanders reported that their cavian strain infected guinea pigs as easily after intravenous as after intracerebral injection.

experiments leave no doubt that after injection into a nerve or subcutaneous tissue the virus reaches the central nervous system by the neural route.

In view of the strictly neurotropic character of the virus, however, it can hardly be assumed that after subcutaneous injection appreciable amounts reach the blood. In the course of the experimental disease it has been found in the blood or organs other than the brain only exceptionally, and the rare positive findings may possibly be explained by centrifugal transmission of the virus along the nerves (Bertarelli; Remlinger and Bailly). After injection into the subcutaneous tissue the virus therefore has no opportunity of reaching the capillaries of the central nervous system.

Pasteur reported that animals can be infected with the virus of rabies by the intravenous route. The results of subsequent investigators were conflicting. Recent experiments of Remlinger and Bailly, Schweinburg, Panisset and Deschamps, and Hurst, however, leave no doubt that with sufficiently virulent strains and adequate dosage the intravenous injection leads to positive results in a considerable proportion of animals.

Whether the virus after intravenous injection reaches the central nervous system directly by way of the capillaries is a much debated question. Hurst found that removal of the olfactory lobes did not prevent infection by the intravenous route. Since, however, the virus may reach the central nervous system by any peripheral nerve, this negative result is obviously of little significance. Thus far no crucial experiments have been reported which would answer the question definitely. Some pertinent observations, however, may be mentioned. The fact that many workers obtained negative results with intravenous injections and the further fact that even after inoculation of the largest doses of the most virulent strains not all of the animals had rabies is difficult to reconcile with the assumption that the virus reaches the central nervous system directly from the blood stream. Moreover, a comparison of the incubation periods following intracerebral and intravenous injection of the virus (Schweinburg, 1932) tends to corroborate one's doubts. With the first route the average period of incubation in three experiments was nine and three-tenths days; with the second route in five experiments it was thirty-one and four-tenths days. This significant difference also would be difficult to understand if the virus reached the central nervous system directly from the blood, whereas it is readily explained if the virus leaves the vascular system and is taken up from the tissues by nerve endings.

Finally Schweinburg (1932) a few hours after injecting the virus intravenously attempted to identify it in various organs. He found that whereas the spleen contained the virus in 9 of 25 experiments the



brain gave negative results throughout. At first sight this experiment appears conclusive. Schweinburg himself, however, pointed to the well known fact that even after intracerebral injection some viruses cannot be identified in the brain for many hours after their injection.

As may be seen from this summary, the question whether the virus of rabies reaches the central nervous system directly from the blood cannot yet be answered with certainty. There exists, however, some presumptive evidence that it does not.

*Borna Disease.*—Zwick, Seifried and Witte (1929) and Nicolau and Galloway found that Borna disease can be produced in rabbits by injecting the virus into the sciatic nerve or into the brachial plexus. After injection into the central nervous system the virus was found in the peripheral nerves (in larger quantities in the proximal than in the distal parts). The neural transport of the virus therefore seems definite. It is questionable whether after peripheral injection the virus can be identified in the blood. Ernst and Hahn obtained positive results, while the experiments of Zwick, Seifried and Witte (1926) and of Nicolau and Galloway gave negative results. The latter authors were unable to infect rabbits by the intravenous route. The former obtained positive results only when the injections were repeated four times. An important observation was reported by Zwick, Seifried and Witte (1929). They found that rabbits could not be infected by the intracutaneous route. The results were usually positive, however, if serum or saline solution was injected simultaneously into the brain. These findings seem to indicate that after intracutaneous injection small quantities of virus reach the blood stream but are unable to pass the normal capillaries of the central nervous system. If these capillaries are damaged, however, infection occurs. If one compares this result with the difficulty with which rabbits are infected by the intravenous injection of large quantities of virus, it becomes unlikely that undamaged capillaries of the central nervous system are permeable to the virus of Borna disease.

*Herpes Simplex.*—The careful histologic investigations of Goodpasture and Teague, Marinesco and Draganesco, and Levaditi and Haber have shown that after intracorneal inoculation the virus of herpes simplex reaches the brain by way of the sensory portion of the fifth cranial nerve. Goodpasture and Teague injected the virus into the masseter muscle, the vitreous humor, the trachea, the skin, striated muscles, the liver, the spleen, the adrenal glands, the peritoneum and the ovaries and found histologic lesions and inclusion bodies only in the central pathways of the nerves that innervated the injected areas. These experiments leave no doubt that after injection into any kind of tissue the virus reaches the central nervous system exclusively by neural pathways.

It is certain, however, that with this mode of inoculation the virus does not appear in the blood with any regularity. On the contrary,

the experiments in which the virus could be identified in the blood have been rather rare exceptions.

Whether the virus of herpes simplex reaches the central nervous system directly from the blood, therefore, can be decided only by means of intravenous injections. The reports in the literature concerning this method of inoculation are conflicting. Many investigators obtained entirely negative results. There is no doubt, however, that with highly virulent strains encephalitis or myelitis has been produced by intravenous injection, although even under these conditions infection did not occur in all of the animals. Levaditi therefore assumed that the positive results are explained by the well known fact that approximately 25 per cent of rabbits suffer from spontaneous encephalitis (*Encephalitozoon cuniculi*) which renders the cerebral capillaries permeable to the virus. Doerr and Hallauer, however, expressed the belief that even in normal animals the virus reaches the central nervous system directly from the blood. It seems to me that the following observations are difficult to reconcile with this belief.

After injection of the virus into the brain the period of incubation for encephalitis is from three to seven days. After intravascular injection, however, Doerr and Zdansky found an incubation period of sixteen to twenty days and Teissier, Gastinel and Reilly one of thirteen to thirty days. Moreover, Doerr and Hallauer found the period of incubation to be the same (five to eleven days) whether the virus was injected into the vascular system or into the pads of the hindlegs of guinea pigs. Since in the latter case the virus reaches the central nervous system exclusively by way of the peripheral nerves, one would expect the period of incubation to be shorter after intravenous injection if the virus reached the central nervous system directly from the blood.

Experiments by Magrassi with a peculiar strain of the virus of herpes simplex are of some significance. While encephalitis developed in the animals after intravenous injection of the ordinary strains, this strain produced only myelitis. Since it was encephalitogenic by the intracerebral route, the peculiar localization of the virus in the lumbar cord would be difficult to understand if it reached the central nervous system directly from the blood. The result would be more plausibly explained by assuming that various strains have predilections for different nerves. Doerr and Hallauer based their opinion that the virus reaches the central nervous system directly from the blood on an interesting experiment. Strain M, which after intravenous injection produced only myelitis, was injected into the distal stump of a transected carotid artery. Of 5 rabbits given this type of injection, 3 showed encephalitis whereas 2 showed symptoms of myelitis. The authors concluded that in the first 3 animals virus had reached the brain directly by way of the cerebral capillaries.

It seems to me, however, that this experiment when correctly interpreted is important evidence for the indirect transmission of the virus of herpes simplex from the blood to the central nervous system. Friedemann and Elkeles showed in experiments with vitally staining dyes that injection into the ramifications of the internal carotid artery through the distal stump of the artery is impossible unless the pressure of injection is exaggerated. The pressure in the circle of Willis is so high that even after very rapid injection none of the injected dye reaches the cerebral arteries. The injected fluid finds its way entirely into the external carotid artery. Since Doerr and Hallauer emphasized that they injected the suspension slowly, it is almost certain that in their experiments no virus reached the cerebral capillaries. The fact that encephalitis developed in 3 of the animals can be explained only by assuming that the virus left the capillary bed of the external carotid artery and reached the brain by way of the cranial nerves. The existing experimental evidence tends to show that the virus of herpes simplex is unable to reach the central nervous system directly from the blood.

*Pseudorabies.*—That the virus of pseudorabies reaches the central nervous system by way of the peripheral nerves has been shown by a variety of experiments. Hurst (1933, 1934), using rabbits, injected the virus simultaneously in the neighborhood of the ear, the flank and the foot. Itching, characteristic of this disease, began in the region of the ear, started considerably later in the flank and was not observed in the foot because of the early death of the animal. Apparently the onset of this symptom was determined by the length of the nerve pathway. After the virus had been injected into the flank, histologic lesions (inflammation and inclusion bodies) were found only in the corresponding nerve, spinal ganglions and posterior horn cells. The virus could be identified in this part of the cord while the remainder of the central nervous system was still virus free.

Sabin inoculated the virus intranasally and found histologic lesions confined to the sensory division of the fifth cranial nerve, the parasympathetic and the sympathetic nerves. No lesions were found in the olfactory nerve and its central connections. This specific distribution of the lesions over certain neurons would obviously be impossible if at the same time the virus reached the brain by the vascular route. This conclusion is important since at least the Aujesky strain of the virus of pseudorabies invades the blood early in the disease and multiplies rapidly in it. The experiments with pseudorabies therefore show clearly that this virus is unable to reach the central nervous system directly from the blood. Experiments of Hurst (1936) on monkeys strengthen this conclusion. This species is readily infected by the intracerebral injection of the virus, while the intramuscular and the intravenous injection are entirely innocuous.

*Virus B.*—The investigations of Sabin have shown conclusively that virus B when injected into the tissues reaches the central nervous system solely via the peripheral nerves. In rabbits the hindlegs became paralyzed whether the virus was injected into the skin, the muscles of the hindlegs, the peritoneum or the testicles. After corneal inoculation encephalitis developed. After the virus had been injected into the skin of the abdomen, the pooled lumbar, dorsal and cervical cords contained five hundred times more virus than the frontal lobes of the brain.

No virus could be identified in the blood after peripheral injection. After intravenous injection all four extremities became paralyzed. The existing experimental evidence is not sufficient to enable one to decide whether or not virus B reaches the central nervous system directly from the circulation.

*St. Louis Encephalitis.*—Webster and Clow showed that mice could be infected with the virus of St. Louis encephalitis by the intranasal route. Twenty-four to forty-eight hours subsequent to the inoculation, virus was found either in the olfactory bulb alone or in this structure and the piriform lobe. These experiments showed conclusively that after intranasal inoculation the virus of St. Louis encephalitis reaches the brain exclusively through the olfactory nerve. Only occasionally could the virus be identified in the blood after intranasal inoculation, but its constant presence in the spleen indicated that it had entered the blood at some stage of the infection.

Even  $10^6$  cerebral minimal lethal doses given intraperitoneally failed to produce encephalitis, although ten minutes, twenty minutes and one and three hours after the injection the blood contained large amounts of the virus (Webster). When, however, the cerebral capillaries were damaged by the intracerebral injection of sterile solutions of starch, encephalitis developed after intraperitoneal injection of the virus. (King recently reported that he could not reproduce these findings.) The experiments of Webster and Clow are very complete in showing that the virus of St. Louis encephalitis is unable to reach the central nervous system from the blood.

*Louping Ill.*—In the case of the virus of louping ill, the question is complicated by the fact that the genesis of the disease apparently is not entirely identical in different animal species. Hardly any investigations pertinent to the problem have been carried out in sheep, the natural host. Galloway and Perdrau infected monkeys by the intranasal route. Since with the exception of 1 animal no virus was found in the blood or visceral organs, they assumed that infection had taken place by neural routes. No definite pathway, however, could be demonstrated by identifying the virus in various parts of the brain. Two monkeys were given large doses intravenously. One animal remained in perfect health although virus was present in the blood for at least six days. The other

monkey became sick and was killed after forty-eight hours. Virus was identified in the blood, the spleen, the lungs and the mesenteric glands but not in the brain. These experiments, although few, seem to indicate that the virus of louping ill is unable to reach the central nervous system directly from the blood.

Burnet and Lush reported important experiments on mice. These animals were readily infected by the intranasal route, and on the second, third and fourth days the virus was found only in the olfactory bulb. Since Fite and Webster reported that virus was present in the blood from the second to the sixth day after intranasal instillation, the results of Burnet and Lush suggest that the virus does not reach the brain directly from the blood. Furthermore, after the intraperitoneal injection of large doses 90 per cent of the animals came down with encephalitis, but three to four days after the injection the virus was found almost exclusively in the olfactory bulbs. By treating the nasal mucous membrane with zinc sulfate, tannic acid or alum the survival time was considerably prolonged. These experiments tend to show that after intraperitoneal injection the virus of louping ill is excreted on the nasal mucosa and carried to the brain by way of the olfactory nerve.

*Neurotropic Strain of Yellow Fever.*—Findlay and Clarke (1935) found that monkeys and mice are susceptible to intranasal inoculation of the neurotropic strain of the virus of yellow fever. In the first stage of infection the virus and histologic lesions were found only in the olfactory bulb and the forebrain. Treatment of the nasal mucous membrane with chemicals gave considerable protection in mice (Findlay and Mahaffy). Since in the monkey, according to Findlay and Clarke (1935), large amounts of virus are found in the blood after intranasal inoculation, the results of Findlay and his colleagues show that the neurotropic strain of the virus of yellow fever is unable to reach the central nervous system directly from the blood. This conclusion is strengthened by the fact that with rare exceptions adult mice (Theiler) and monkeys cannot be infected by the intraperitoneal or by the subcutaneous route. According to Sawyer and Lloyd, however, infection occurs if the cerebral capillaries are damaged by an intracerebral injection of a solution of starch. It is interesting to note that according to Theiler and Findlay and Clarke (1935) very young mice and hedgehogs can be infected by the subcutaneous and by the intraperitoneal route.

*Vesicular Stomatitis.*—Sabin and Olitsky (1937 a,b,c; 1938 d,e) have shown that the virus of vesicular stomatitis when injected into various tissues of 15 day old mice gains access to the central nervous system exclusively by neural routes. After intranasal inoculation it appeared first in the rhinencephalon; after intraocular injection, in the diencephalon and the mesencephalon, and after injection into the muscles of the hindlegs, in the lumbar cord. The pathways of the virus were

determined more precisely with histologic methods. After intranasal inoculation lesions were found in the olfactory tracts; after intraocular injection, in the optic tract. In both cases the lesions extended to the cortical areas of these nerves. One year old mice could not be infected by the intranasal, the intraocular or the intramuscular route although the lethal dose by the intracerebral route was the same for young and old animals.

The authors emphasized that no systemic infection occurs after intranasal or intraocular inoculation. In young mice injection of the virus into the vein of the tail produced paralysis of the hindlegs. Sabin and Olitsky concluded that in this case the virus reached the cord by neural pathways, probably after having escaped into the subcutaneous tissue. In their entirety the experiments of Sabin and Olitsky indicate that the virus of vesicular stomatitis is unable to pass the capillaries of the central nervous system.

*Equine Encephalomyelitis.*—The interpretation of the experiments with the virus of equine encephalomyelitis meets with particular difficulties since the results are apparently determined by the species and the age of the experimental animal, possibly by the type of virus (Eastern or Western strain) and by the route of injection. The findings in the mouse are relatively clear. After intranasal instillation histologic lesions were observed only in the olfactory tract, and after intraocular injection, exclusively in the optic tract (Sabin, 1938; Sabin and Olitsky, 1937, 1938). When Eastern virus was inoculated into the muscles of the hindlegs of mice 15 to 21 days old flaccid paralysis of these limbs developed in 5 per cent of the animals, indicating that the virus had reached the central nervous system by way of the local nerve. All other animals died of encephalitis. Most of the mice, however, escaped involvement of the central nervous system if the nasal mucosa was treated with chemicals prior to the intramuscular injection of the virus. In the few animals in which encephalitis developed despite this treatment lesions were found in the cord and the medulla oblongata (spread via nerves supporting muscle), the central pathways of the auditory and vestibular nerves and the medullary nucleus of the seventh nerve. The experiments of Sabin and Olitsky in mice therefore tend to show that after intranasal and intraocular injection and only in exceptional cases after intramuscular injection the virus of equine encephalomyelitis reaches the central nervous system exclusively by peripheral nerves from the inoculated site. Since after intramuscular injection the virus invades the blood stream in most of the animals and may multiply in it and circulate for a few (one to two) days, it must be concluded that in the mouse the virus of equine encephalomyelitis is unable to reach the central nervous system through the capillary endothelium. All these experiments were carried out in young mice. Old mice are resistant to the intramuscular injection of the virus although when inoculated by the intracerebral route young and

old mice are equally susceptible and although in both age groups the virus circulates in the blood in the same phase. It is obvious, therefore, that in old mice the virus does not reach the central nervous system directly from the blood.

More difficulties are encountered in the interpretation of the experiments on guinea pigs. Hurst and also Howitt found that these animals can readily be infected by the intranasal route with Western virus. After injection of the virus into the muscles of the hindleg, histologic lesions were found predominantly in the frontal lobe of the brain. However, encephalitis was not prevented by removal of the olfactory bulbs and the anterior parts of the olfactory tracts. Hurst therefore assumed that the operation as such damaged the blood-brain barrier and thus, despite interruption of the normal olfactory pathway, made the brain accessible to the virus. In view of the fact, however, that the same operation prevents poliomyelitis after intravenous injection of large doses of this virus, the explanation of Hurst can hardly be accepted. It appears as if the virus of equine encephalomyelitis does reach the central nervous system by way of the olfactory nerve but may also use other routes. As a matter of fact, Sabin and Olitsky (1938 a) and King (1938) found that after injection of mouse passage Eastern virus into the hindleg lesions were scattered all over the central nervous system especially the neopallial cortex, which is rarely involved in the mouse. Lesions were present near damaged blood vessels. The endothelium of the capillaries was swollen and proliferated and contained inclusion bodies. The histologic observations of Sabin and Olitsky (1938 a) as well as those of King indicate that the Eastern virus grows through the vessels and involves the nerve cells around them (compare also Hurst). Old guinea pigs given  $10^7$  or fewer mouse cerebral lethal doses are resistant (Sabin and Olitsky, 1938 b). In an exceptional guinea pig which became infected, the lesions were not scattered but corresponded to the innervation of the leg into which the virus was injected.

The rather complicated results may be briefly summarized as follows: In young and old mice, and in old guinea pigs under special conditions, the virus of equine encephalomyelitis does not reach the central nervous system directly from the blood. In young guinea pigs the virus grows through the blood vessels and reaches nerve tissues by this route. In this connection Dr. P. K. Olitsky, of the Rockefeller Institute for Medical Research, gave valuable advice and suggestions.

*Canine Distemper.*—Experiments of De Monbreun seem to indicate that the virus of distemper reaches the central nervous system in dogs in essentially the same way as does that of equine encephalomyelitis in young guinea pigs.

*Fowl Plague.*—The virus of fowl plague may be included in this discussion although, according to the nomenclature of Hurst,<sup>3</sup> it is pantropic rather than neurotropic in the strict sense of the word. Doerr and Seidenberg (1932-1933), however, have shown that in the chicken the brain at the time of death contains much more virus than can be accounted for by the blood content of the organ. In view of the fact that virulent strains usually kill chickens within thirty to forty-eight hours it is unlikely that the virus reaches the brain by neural pathways. Moreover, in the guinea pig and the mouse the virus was identified in the brain three hours after its intravenous injection although it had disappeared from the blood within one to two hours. Doerr therefore assumed that the virus of fowl plague reaches the central nervous system directly from the blood.

It is of great interest that the results differed with other strains of the virus and in other species. Working with the less virulent Egyptian strain, Lagrange found that the course of the disease was much more protracted. In the second stage the virus was found in the brain, but in the first stage the brain was virus free for several days, although large amounts were found in the blood. Lagrange concluded from these experiments that the cerebral capillaries are impermeable to the Egyptian strain of the virus of fowl plague. Doerr and Seidenberg (1931-1932) were unable to identify the virus in the brains of rabbits into which it was injected intravenously. Theiler,<sup>4</sup> Kleine and Moellers found that adult geese could not be infected by the subcutaneous or the intramuscular route, although the virus was highly pathogenic if given intracerebrally. These findings are very interesting from the point of view of the neurotropism of viruses. In this context one may confine oneself to stating that certain strains of the virus of fowl plague in certain animal species apparently are able to pass the capillaries of the central nervous system.

*Summary of Recorded Results.*—The results of the experiments discussed in this review may be summarized as follows: The blood-brain barrier is impermeable to the majority of neurotropic viruses. The evidence to that effect is conclusive or at least highly suggestive for the viruses of poliomyelitis, rabies, Borna disease, herpes simplex, louping ill, pseudorabies, St. Louis encephalitis, vesicular stomatitis, the neurotropic strain of yellow fever and for equine encephalomyelitis in mice and old guinea pigs. Virus B. when injected subcutaneously also reaches the central nervous system exclusively by the neural route, but for the time being it is impossible to ascertain that it is unable to pass the blood-brain barrier.

3. The reference is to Hurst (1936) under "General Literature" in the bibliography.

4. The reference is to Theiler under "Neurotropic Stain of Yellow Fever" in the bibliography.



In young guinea pigs the virus of equine encephalomyelitis passes directly through the walls of the cerebral capillaries. But this passage cannot be considered a physicochemical process. It is mediated by the multiplication of the virus within the endothelial cells. The same mechanism is probably operative in the case of the virus of canine distemper.

The only virus to which the blood-brain barrier is permeable in a physicochemical sense is the pantropic virus of fowl plague. This, however, holds true only for highly virulent strains in chickens, mice and guinea pigs.

#### ELECTROCHEMICAL ASPECTS OF THE PROBLEM

It has been shown in the preceding section that in a physicochemical sense the capillaries of the central nervous system are probably impermeable to all neurotropic viruses with the exception of the virus of fowl plague. In looking for an explanation of these findings, one must in the first place consider the factor of size. Neurotropic viruses are corpuscular elements, and it might appear plausible that this factor prevents them from passing the capillary membrane. Quantitative considerations, however, cast some doubt on the validity of this assumption. As has been shown elsewhere, the capillaries of the central nervous system are permeable to antibodies. The elementary bodies of some viruses (those of poliomyelitis, louping ill, foot and mouth disease), however, are so small that they hardly exceed in size large protein molecules. Moreover, it has been seen that the capillaries of the central nervous system are permeable to the virus of fowl plague, the elementary bodies of which are not even particularly small.

In my review on the blood-brain barrier I showed that the ability of aniline dyes, toxins, antibodies and drugs to pass the capillaries of the central nervous system is determined by their electrical charge. The question naturally presents itself whether the same rule holds true for neurotropic viruses. A discussion of this question is complicated by the rather conflicting evidence concerning the electrical charge of neurotropic viruses at the hydrogen ion concentration of the blood. Obviously, the results of cataphoretic experiments may be vitiated by technical errors which have not always been taken into consideration. The most important of these errors is the interference of electroendosmotic water currents which shift the isoelectric point to the alkaline side. In the following tables I have recorded, therefore, only results obtained with the technics of Todd and Michaelis in which this error is as far as possible eliminated. Concerning the neurotropic viruses listed in the accompanying table reliable data are available in the literature.

As may be seen from the table, the neurotropic viruses thus far investigated carry a negative charge at the hydrogen ion concentration of

the blood. This would be in keeping with their inability to pass the capillaries of the central nervous system. In view of the fact, however, that most biologic products are negatively charged it may be questioned whether this agreement between experimental facts and theoretic expectation is more than a coincidence. The only virus known to pass through the capillaries of the central nervous system is the pantropic strain of the virus of fowl plague studied by Doerr and Seidenberg. The electrical charge of this virus, therefore, is of the greatest theoretic interest. I have not been able to find cataphoretic experiments with this strain of virus reported in the literature. Dr. A. Todd, of the National Institute for Medical Research, London, however, informed me about his own unpublished experiments. In contradistinction to all other viruses, the virus of fowl plague went neither to the anode nor to the cathode. Obviously, this virus is isoelectric at the hydrogen ion concentration of the blood. Experiments of Doerr and Gold point in the same direction.

*Cataphoretic Experiments with Neurotropic Viruses*

Virus	$pH$	Charge	Author
Louping ill.....	7.3	Negative	Lépine (1931)
Borna disease.....	6.6-7.4	Negative	Nicolau and Kopciowska (1931)
Yellow fever.....	5.2-7.0	Negative	Hindle and Findlay
Herpes simplex.....	5.3-7.8	Negative	Nicolau and Kopciowska (1930 <sup>a,b</sup> )
Rabies.....	5.8-7.4	Negative	Nicolau and Kopciowska (1930 <sup>c</sup> )
Polioomyelitis.....	6.9-8.0	Negative	Levaditi and Lépine
Equine encephalomyelitis (Eastern strain)	Above 4.1	Negative	Finkelstein, Marx, Bridgers and Beard
Vesicular stomatitis.....	6.6-8.4	Negative	Oltzky and Cox (personal communication)

They found that the virus of fowl plague is much more strongly absorbed by the electronegative charcoal than are other viruses and bacteriophages. Since Friedemann and Elkeles have shown that the isoelectric toxin of lamb dysentery passes through the cerebral capillaries, the findings of Todd tend to show that the ability of viruses to pass these capillaries may, indeed, be determined by their electric charge. It has been seen that, according to Lagrange, the Egyptian strain of the virus of fowl plague is unable to reach the central nervous system through the capillary walls. It is of great interest that another French author, Lépine (1930), reported a negative charge for the virus of fowl plague over a range of  $pH$  6.2 to 8.2.

The experimental data concerning the electrical charge of viruses are not yet numerous enough to arrive at final conclusions. As far as the evidence goes, it is certainly not at variance with the assumption that in conformity with the results obtained with aniline dyes, toxins, antibodies and drugs, the ability of viruses to pass the capillaries may be related to their electrical charge. At least this hypothesis lends new significance to cataphoretic experiments with viruses. It is to be hoped

that in the future a more extensive experimental material obtained with a uniform and impeccable technic will be available.

It seems that the electrochemical concept of capillary permeability gives the only satisfactory explanation of the experimental findings reviewed in this paper. Obviously, the propagation of neurotropic viruses in the paths of certain neurons becomes understandable only if one considers the blood-brain barrier as completely impermeable to these viruses. Even a few elementary bodies passing through the capillary walls would infect the central nervous system and mar the clear picture of axonal spread. The difficulty in explaining this complete impermeability of the blood-brain barrier to neurotropic viruses along mechanical lines has induced Doerr to discard the whole theory of the blood-brain barrier. Obviously, this difficulty does not exist any longer once observers become reconciled to the idea that the permeability of the capillaries of the central nervous system is regulated by electrical forces.

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## Notes and News

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**Appointments.**—Herbert S. Breyfogle, a fellow in legal medicine at Harvard Medical School, has been appointed instructor in pathology at the Washington University School of Medicine and pathologist to the St. Louis County Hospital, effective May 1. Dr. Breyfogle will serve as pathologist to the coroner of St. Louis County.

Granville A. Bennett, of the department of pathology at Harvard Medical School, has been appointed professor of pathology and bacteriology at the school of medicine of the Tulane University of Louisiana, New Orleans.

**Awards.**—Vincent du Vigneaud, professor of biochemistry at the Cornell University Medical College, has been given the Mead Johnson & Company award of \$1,000 for research on the vitamins of the B complex, in recognition of his work on biotin.

The Civic Medal awarded annually by the Rochester Museum Association was presented on May 13 to George H. Whipple, dean of the University of Rochester School of Medicine and Dentistry.

The Theobald Smith Award of \$1,000 and a bronze medal of the American Association for the Advancement of Science, established by Eli Lilly & Company in 1935, has been given to Sidney C. Madden, assistant professor of pathology at the school of medicine and dentistry of the University of Rochester, in recognition of his work on plasma proteins.

**National Academy of Sciences.**—Warfield T. Longcope, of the department of medicine at Johns Hopkins University, and O. H. Robertson, of the department of medicine at the University of Chicago, have been elected members of the Academy.

**Deaths.**—Harry Gideon Wells died April 26 at the age of 67 years. James Ewing died May 16 at the age of 76 years. Obituary sketches of Dr. Wells and Dr. Ewing will appear in a forthcoming number.

**Hormones.**—A symposium on hormones will be held at Gibson Island July 19 to July 23 in connection with this summer's conferences under the auspices of the American Association for the Advancement of Science.

**Foundation for Infantile Paralysis.**—This foundation has made long term grants for the study of infantile paralysis and related diseases as follows: John Hopkins University, \$300,000; Yale University, \$150,000; University of Michigan, \$230,000. A bibliography of the scientific literature on infantile paralysis is in course of preparation.

# Books Received

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**TWENTY YEARS OF MEDICAL RESEARCH.** Dorothy White Nicolson. Pp. 97. New York: Medical Research Committee, National Tuberculosis Association, 1943.

This is an instructive and comprehensive review of the work of the Committee on Medical Research (William Charles White, chairman) of the National Tuberculosis Association. The director of the association, Kendall Emerson, states in the preface that the outcome of the work here reviewed "proves the effectiveness of the policy adopted from the beginning, a logical selection of topics for study, seeking the expert best qualified to carry forward a special phase of the research, enlisting the interest of universities in providing necessary facilities." The survey and the bibliography of the researches aided by the committee, which clearly justify Dr. Emerson's statement, will be of interest to those who are concerned with cooperative research in general and with research in tuberculosis in particular.

**PROTEIN HORMONES OF THE PITUITARY BODY.** H. B. Van Dyke, Bacon F. Chow, Vincent du Vigneaud, H. L. Fevold, George W. Irving Jr., C. N. H. Long, Theodore Shedlovsky and Abraham White. *Annals of the New York Academy of Sciences.* Volume 43. Pp. 253-426. New York: New York Academy of Sciences, 1943.

This volume consists of a valuable series of papers presented at a conference on the protein hormones of the pituitary gland in the section of physics and chemistry of the New York Academy of Sciences. The authors and topics of the papers follow: H. B. Van Dyke, introduction; Theodore Shedlovsky, criteria of purity of proteins; George W. Irving Jr. and Vincent du Vigneaud, hormones of the posterior lobe of the pituitary gland; Bacon F. Chow, the chemistry of "thylakentrin," the follicle-stimulating hormone of the anterior lobe of the pituitary gland; H. L. Fevold, the luteinizing hormone of the anterior lobe of the pituitary gland; Abraham White, the lactogenic hormone and mammogen; C. N. H. Long, the growth-promoting and metabolic hormones of the anterior lobe of the pituitary gland.

**CLINICAL SIGNIFICANCE OF THE BLOOD IN TUBERCULOSIS.** Gulli Lindh Muller, M.D., pathologist and director of laboratory, New England Hospital for Women and Children, Boston; formerly pathologist, Rutland State Sanatorium, Rutland, Mass. Pp. 516. Price \$3.50. New York: The Commonwealth Fund, 1943.

The basis of this book is complete hematologic studies of 1,000 cases of tuberculosis (6,819 complete examinations) coupled with a correlation of the literature on the blood in tuberculosis. The book is divided into five parts: the physiology of the blood-forming organs and the cellular response to the tubercle bacillus; changes in the circulating blood in tuberculosis; the sedimentation rate; clinical and hematologic data as measures of the constitutional reaction; the effect of therapeutic measures, exercise and certain complications on the hematologic picture; methods for the examination of the blood. There are numerous tables and charts. The book is an important addition to the literature in its field.

**OUTLINE OF ROENTGEN DIAGNOSIS: AN ORIENTATION IN THE BASIC PRINCIPLES OF DIAGNOSIS BY THE ROENTGEN METHOD.** Leo G. Rigler, B.S., M.B., M.D., professor of Radiology, University of Minnesota, Minneapolis. Pp. 196, with 227 figures, presented in drawings and reproductions of roentgenograms. Figures 6 to 51 and 55 to 72 are drawings in an original technic by Jean E. Hirsch. Philadelphia: J. B. Lippincott Company, 1943.

The text has been revised and expanded to include recent advances in the technic, the methods and the scope of roentgen examination, but the size of the book has not been increased. The book will maintain its standing as an excellent outline for the teaching of roentgen diagnosis.

**THE SIGHT SAVER.** C. J. Gerling. Pp. 202. Price \$2. New York: Harvest House, 1943

This book, organized like a dictionary or an encyclopedia, deals comprehensively and accurately with the eye and with conservation of sight. In no sense does it tend to replace the physician or the ophthalmologist but presents tellingly the dangers to vision of fraud and quackery. The language is clear and simple. The book should be widely used.



A MANUAL OF PULMONARY TUBERCULOSIS (PART I) AND AN ATLAS OF THORACIC ROENTGENOLOGY (PART II). David O. N. Lindberg, M.D., F.A.C.P., lecturer on tuberculosis, State University of Iowa College of Medicine; director of roentgenology, State Sanatorium, Iowa Pp. XVI and 219 plus an index, with 189 illustrations, including 145 plates. Price \$6.50. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

Dr. Lindberg has produced a concise, well illustrated description of the diagnosis, the general and the surgical treatment, and the control of pulmonary tuberculosis, supplemented by an atlas (145 figures) of thoracic roentgenology.

LA UROBILINA EN EL ESTADO NORMAL Y PATOLÓGICO. Marcelo Royer. Trabajo del Instituto de fisiología Facultad de ciencias médicas, Buenos Aires. Second edition. Pp. 265, with 43 figures. Buenos Aires: Editor "El Ateneo," 1943.

The first edition was published in 1929, and a French edition appeared in 1930. A full account is given of human urobilin under physiologic and pathologic conditions, its determination, its variations, its elimination and its significance in tests of hepatic function.

CHEMOTHERAPY OF GONOCOCCIC INFECTIONS. Russell D. Herrold, B.S., M.D., associate professor of surgery (urology), University of Illinois College of Medicine, Chicago. Pp. 137. Price \$1. St. Louis: C. V. Mosby Company, 1943.

An important, timely, practical contribution to the effective treatment of gonococcal infections, based on the personal observations of the author.

DICTIONARY OF BIO-CHEMISTRY AND RELATED SUBJECTS. Editor in Chief, William Marias Malisoff, professor of biochemistry at the Polytechnic Institute of Brooklyn. Pp. 579. Price \$7.50. New York: Philosophical Library, 1943.

A GUIDE TO THE PRESERVATION OF LIFE AT SEA AFTER SHIPWRECK. Medical Research Council, Committee on the Care of Shipwrecked Personnel: War Memorandum No. 8. Pp. 21. Price 10 cents. London: His Majesty's Stationery Office, 1943. (The British Library of Information, 30 Rockefeller Plaza, New York.)

SCHOOL OF TROPICAL MEDICINE UNDER THE AUSPICES OF COLUMBIA UNIVERSITY, SAN JUAN, PUERTO RICO. REPORT OF THE DIRECTOR FOR THE YEAR ENDING JUNE 1942. Published by the University of Puerto Rico and Columbia University.

THE NATIONAL FOUNDATION FOR INFANTILE PARALYSIS, INC. ANNUAL REPORT, 1942. Pp. 55. New York: 120 Broadway.

TEN YEARS' PROGRESS IN CANCER RESEARCH. A SYMPOSIUM COMMEMORATING THE TENTH ANNIVERSARY OF THE INTERNATIONAL CANCER RESEARCH FOUNDATION. Pp. 53. Philadelphia: The International Cancer Research Foundation.

HOPE DEFERRED. Jeanette Seletz. Pp. 536. New York: The Macmillan Company, 1943.

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